Supplementary information

Materials & Methods

Materials

Raw whole bovine milk was provided by CARUS Farm from Wageningen University & Research. NuPAGE 4 to 12% Bis-Tris protein gel, NuPAGE LDS sample buffer (4x), NuPAGE sample reducing agent (10X) and NuPAGE MES SDS running buffer (20X) were purchased from Thermo Fisher Scientific. BlueRay prestained protein marker was obtained from Jena Bioscience.

Reducing SDS-PAGE

Reducing SDS-PAGE was performed to determine the protein composition before and after filtration of the intestinal digests. Raw, undigested skim milk was measured as a reference. First, undigested skim milk was diluted seven times with Milli-Q water. Then, 4 µl intestinal digest, filtered intestinal digest or diluted skim milk was mixed with 5 µl 4x LDS sample buffer, 2 µl 10x reducing agent, and 9 µl Milli-Q water. Samples were heated at 70°C for 10 min, and 10 µl of each sample and 4 µl marker were loaded on a 4-12% Bis-Tris polyacrylamide precast gel. Gels were run at 120V for 75 min with MES running buffer, and stained with Coomassie brilliant blue solution for 2h, and destained with 10% ethanol and 7.5% acetic acid in demi water. The gels were scanned with a GS-900 Calibrated Densitometer (Bio-Rad) with Image Lab software (Bio-Rad).

Protein concentration

Protein concentrations of the intestinal digests before and after filtration were measured by DUMAS. Total nitrogen concent of the samples was determined with a DUMAS Flash EA 1112 Protein analyzer (Thermo Fisher Scientific), and nitrogen content was converted to protein contencentration with a conversion factor of 6.38.

2.1 Mucus staining

Mucins produced by Caco-2 and HT29-MTX-E12 cells were stained and visualized by Alcian Blue followed by a PAS staining using either an ethanol or Carnoy’s fixation. Alcian blue stains acidic mucins, while PAS stains neutral mucins.

Fixation in Carnoy’s or ethanol solution

Caco-2 and HT29-MTX cells were co-cultured on inserts in ratio’s 100:0 and 90:10. For each ratio, a duplo set of inserts were seeded as described previously in section 2.8. After 21 days of growth on inserts, the medium was removed by turning the inserts upside down in a Petri dish and droplets still present were removed carefully by pipetting. To one set of inserts (n=1), 200 µl fresh Carnoy's solution (60% ethanol, 30% chloroform, 10% acetic acid) was added and inserts were incubated for 30 min at RT. After removing the fixative by pipetting, 200 µl of PBS was added and inserts were stored at 4°C in a plate until staining, wrapped shut with parafilm to prevent evaporation.

The second set of inserts (n=1) were fixated in ice-cold ethanol (-20°C). Medium was removed as described above and 200µl of ethanol was added and inserts were incubated for 30 min at RT. The

fixative was removed by pipetting and 200 µl of PBS was added. Inserts were stored at 4°C in a plate wrapped shut with parafilm until staining.

Alcian Blue/PAS staining

All stored inserts (fixated either in Carnoy's or ethanol solution) were carefully washed by adding 150 µl distilled water apical and 750 µl basolateral, and removing it by pipetting. Then, Alcian Blue solution (1g Alcian Blue 8GX in 100 ml 3% Acetic Acid Solution, set to pH 2.5) was added to both compartments (apical 150 µL; basolateral 750 µL) and inserts were incubated for 5 min at RT. Inserts were tapped upside down to remove the Alcian Blue staining and washed by dipping twice in demi water. Then, the inserts were thoroughly washed by keeping them under running tap water for 3 min. Inserts were dipped once more in demi water. Subsequently, inserts were tapped dry and transferred to a clean 24-well plate. For all inserts, Alcian Blue staining was followed by periodic acid staining to visualize both acid and neutral mucins in the same insert. Periodic Acid solution 1 (PAS staining kit) was added to both compartments (apical 150 µL; basolateral 750 µL) and inserts were incubated for 10 min at RT. Periodic acid staining was removed by tapping upside down and inserts were again washed as described previously. Inserts were incubated with Schiff's reagent (solution 2; apical 150 µL; basolateral 750 µL) for 15 min at RT. After removing the Schiff's reagent by tapping upside down, inserts were again washed as described previously. To enhance the specificity of the staining, inserts were washed 3 times with sulfite water (10ml of 10% Sodium Bisulfite solution, 10ml of 1M HCl, 200ml tap water) for 2 min. Stained membranes were removed from the inserts using a small scalpel blade and place cells side-up onto a glass slide, and sealed with DPX mounting medium under a cover glass. Slides were kept in the fume hood overnight to harden, covered by aluminum foil.

The slides were analyzed using the widefield light microscope Axioskop with Olympus XC30 camera (Zeiss, Germany). All pictures taken with 10x magnification (lens aperture 0.25) using the CellSens Entry software version 1.16 (Olympus Scientific solutions/ Evident Scientific; Hamburg, Germany). Per insert, 4 images were taken, one from each quartile and representative images are shown in Figure S6.

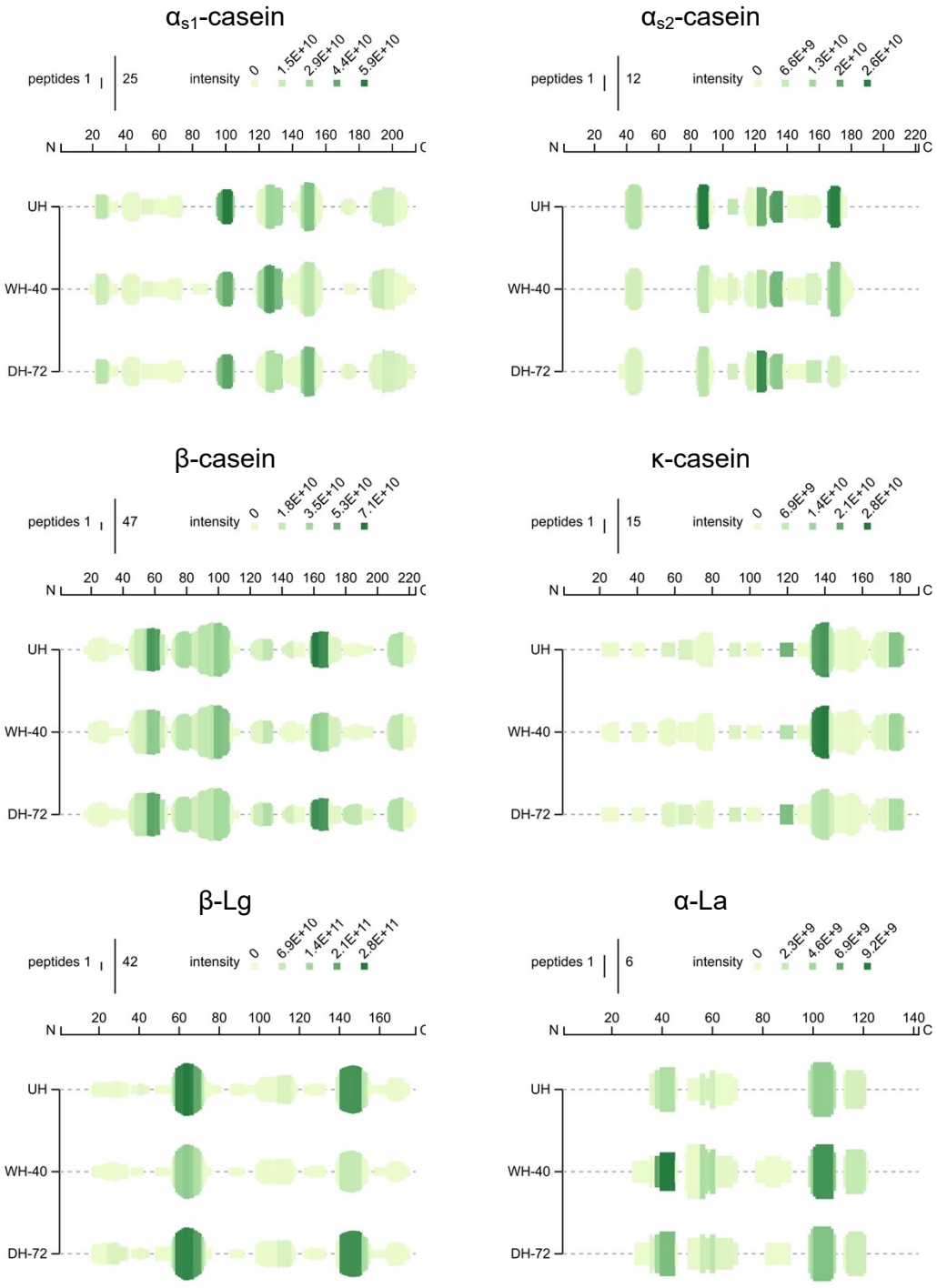
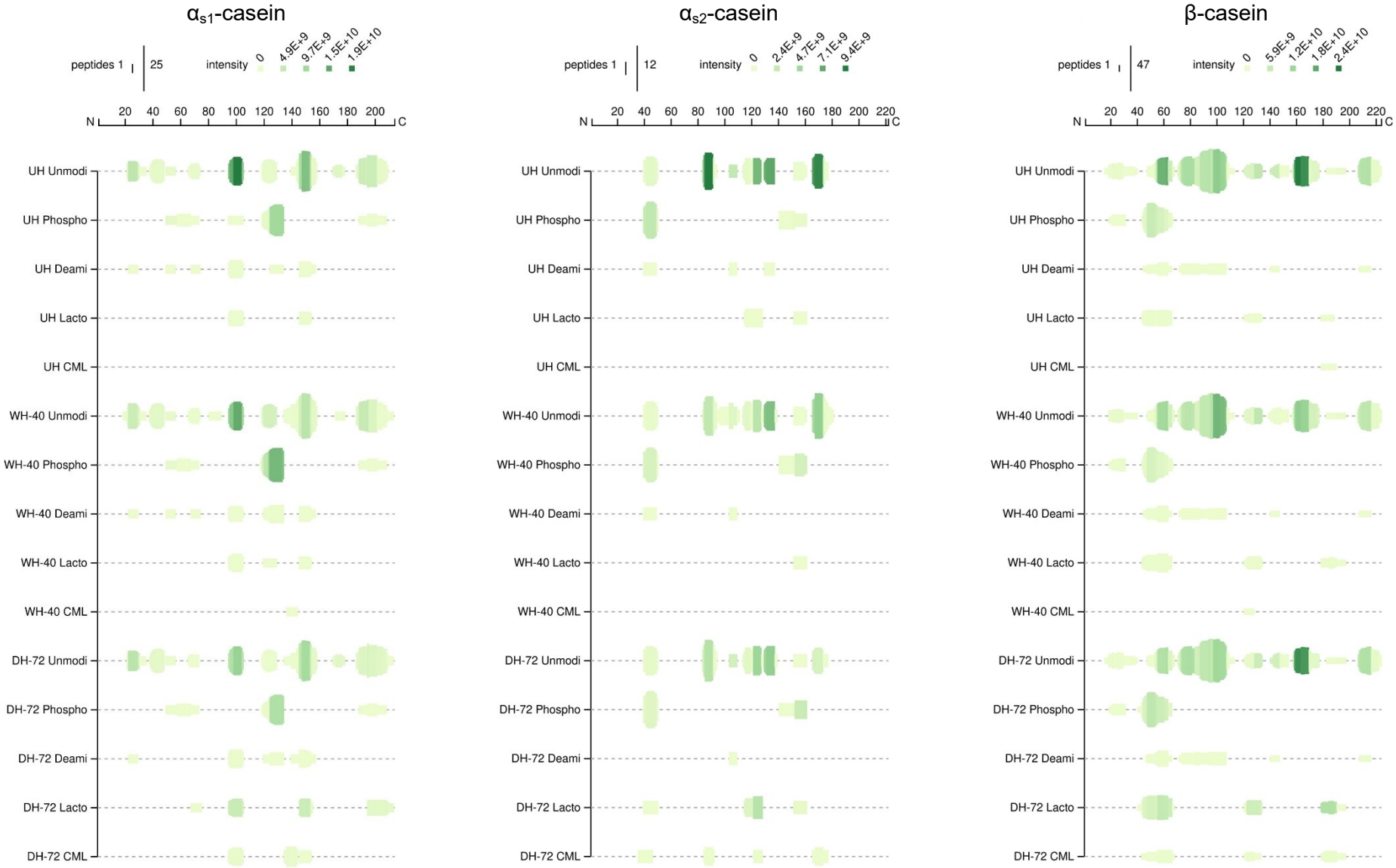


Figure S1: Peptide alignment for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-lactoglobulin (β-Lg), and α-lactalbumin (α-La) detected with LC-MS/MS in the filtered intestinal digests (n=3). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model. The color of the bars represents the sum of peptide intensities that overlap at this position, and the height of the bars represents the number of peptides (peptide count) that overlap at this position. The amino acid position is given including the signal peptides of 15, 15, 15, 21, 16, and 19 amino acids for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-Lg, and α-La, respectively.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N C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
αs2-casein
peptides 1 12
intensity 0 2.4E+9 4.7E+9 7.1E+9 9.4E+9
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N C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
β-casein
peptides 1 47
intensity 0 5.9E+9 1.2E+10 1.8E+10 2.4E+10
20 40 60 80 100 120 140 160 180 200 220
N C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
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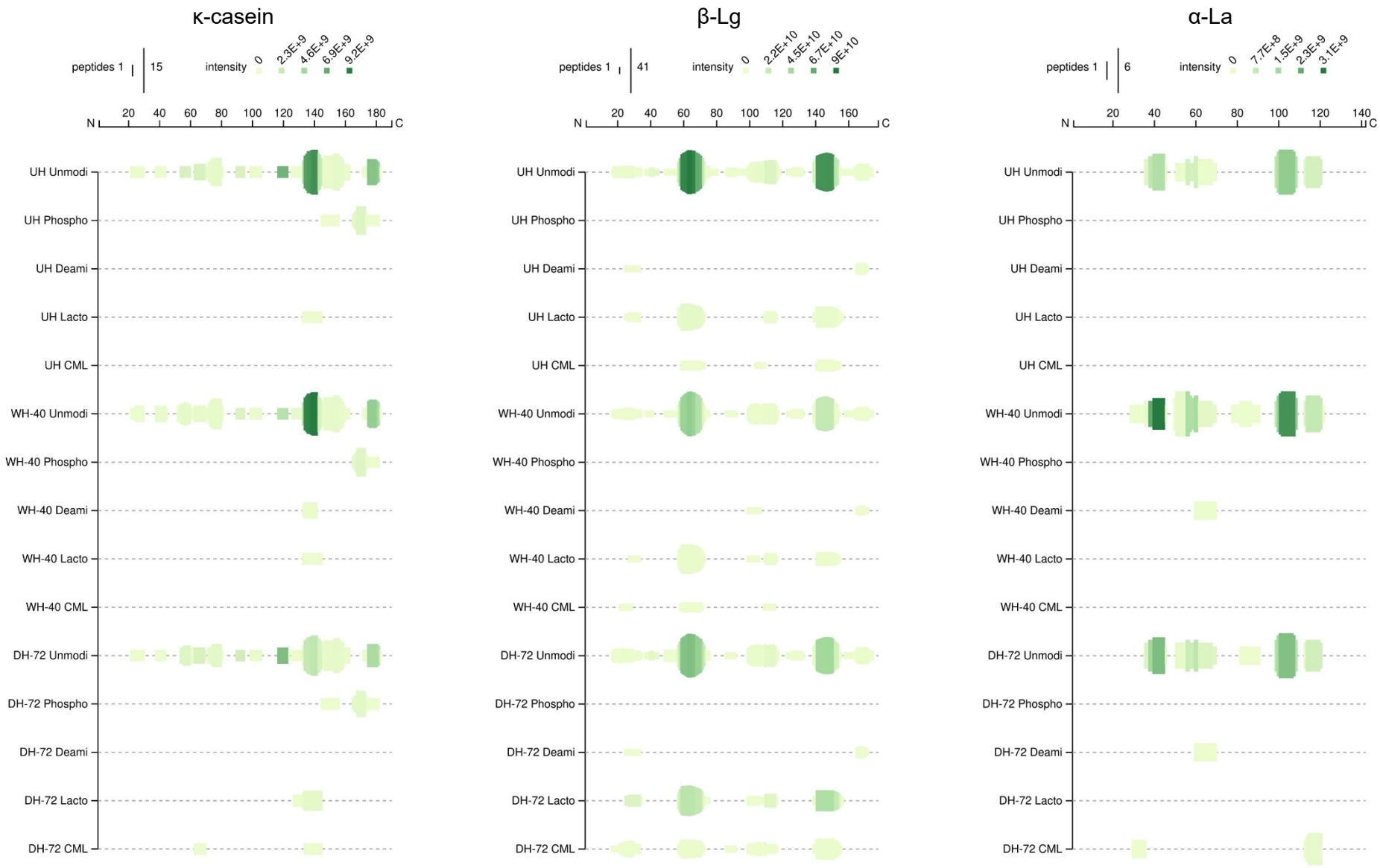
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40
60
80
100
120
140
160
180
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
β-Lg
peptides 1
41
intensity
0
2.2E+10
4.5E+10
6.7E+10
9E+10
N
20
40
60
80
100
120
140
160
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
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DH-72 Lacto
DH-72 CML
α-La
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2.3E+9
3.1E+9
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40
60
80
100
120
140
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML

Figure S2: Peptide alignment for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-lactoglobulin (β-Lg), and α-lactalbumin (α-La) detected with LC-MS/MS in the filtered intestinal digests. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model (n=3). Peptide alignments are shown separately for unmodified (Unmodi), phosphorylated (Phospho), deamidation (Deami) modified, lactosylated (Lacto) and carboxymethyllysine (CML) modified peptides. The color of the bars represents the sum of peptide intensities that overlap at this position, and the height of the bars represents the number of peptides (peptide count) that overlap at this position. The amino acid position is given including the signal peptides of 15, 15, 15, 21, 16, and 19 amino acids for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-Lg, and α-La, respectively.

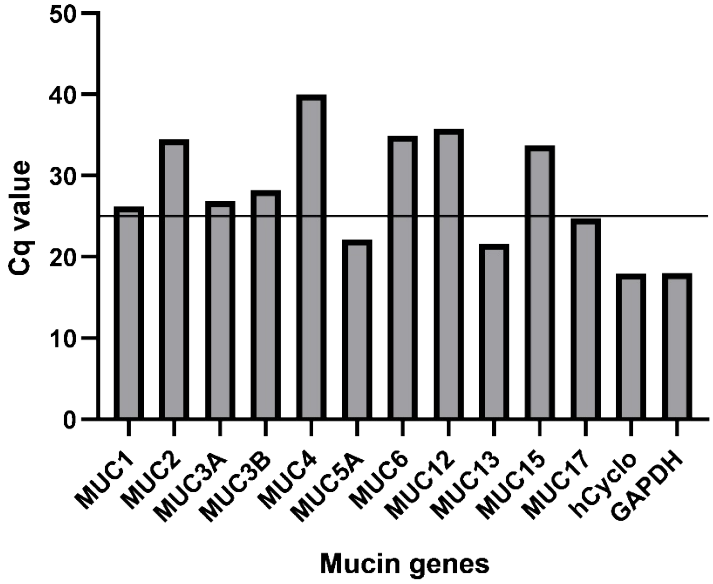


Figure S3: Expression levels of mucin genes measured by qPCR in HT29-MTX-E12 cells (n=1, average of technical duplo). qPCR was performed as described in the materials & methods (section 2.7). Primer details are indicated in Table S1.

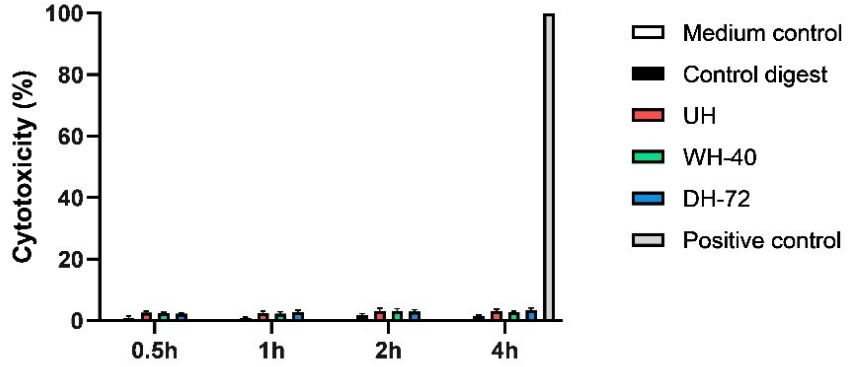
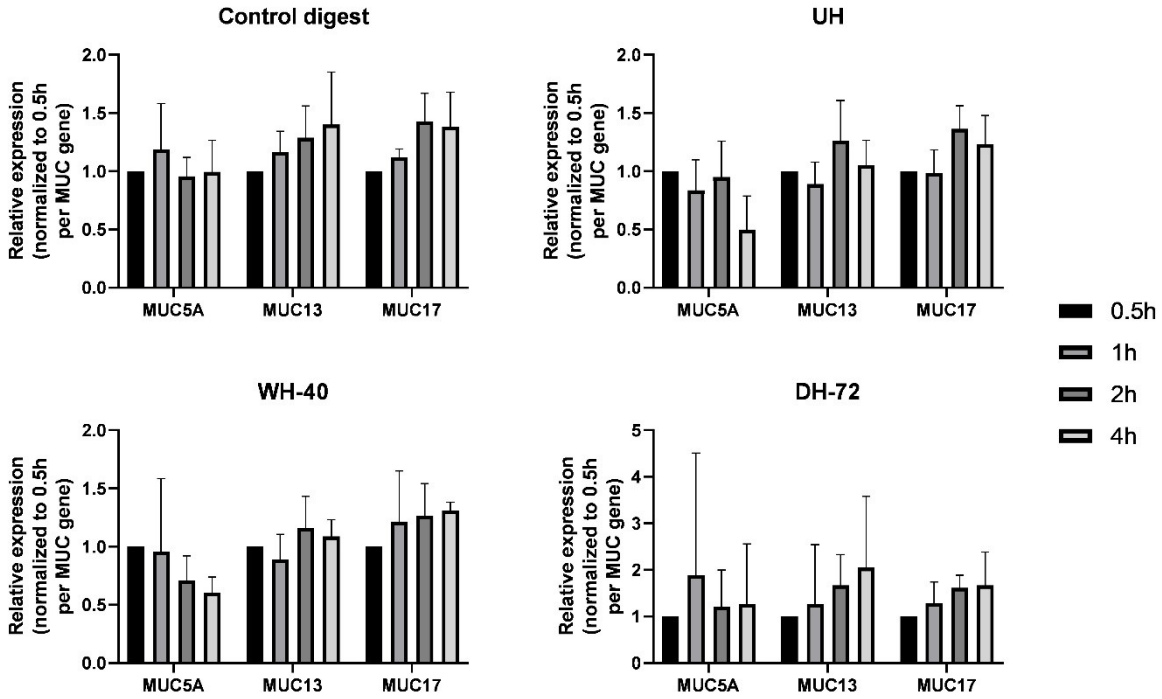


Figure S4: Cytotoxicity effects on HT29-MTX-E12 cells after exposure to the filtered intestinal digests for the mucin gene expression and mucus production experiments as measured with the LDH assay (n=3). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the HT29-MTX-E12 cells for 0.5-4h. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium control was HT29-MTX-E12 cells exposed to DMEM, and was set to 0%. The LDH positive control 2% Triton X100 releases the maximum amount of LDH present within the HT29-MTX-E12 cells, and was set at 100%. Error bars represent standard deviation.

(a)



(b)

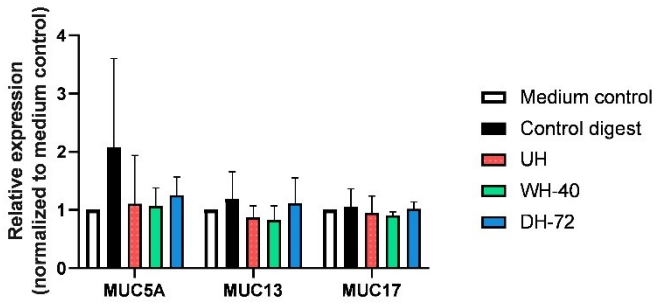


Figure S5: Mucin gene expression after application of the filtered intestinal digests to HT29-MTX-E12 cells (n=3). (a) Mucin gene expression after 0.5, 1, 2, or 4h relative to the 0.5h time point for each MUC gene and digest. (b) Mucin gene expression after 4h relative to the medium control for each MUC gene. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72), and all samples were digested by use of an *in vitro* infant digestion model. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium control were HT29-MTX-E12 cells exposed to Dulbecco's Modified Eagle Medium (DMEM). No statistical differences were found between the medium control, control digest, and milk digest samples. Error bars represent standard deviation.

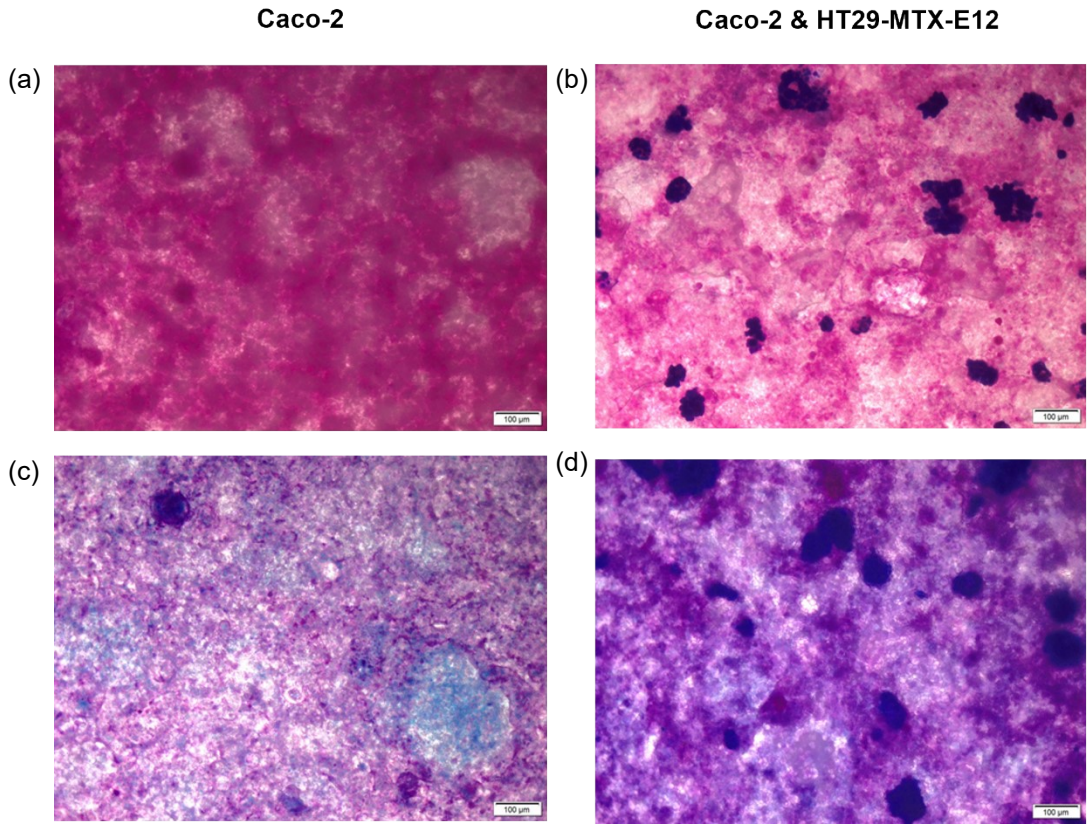
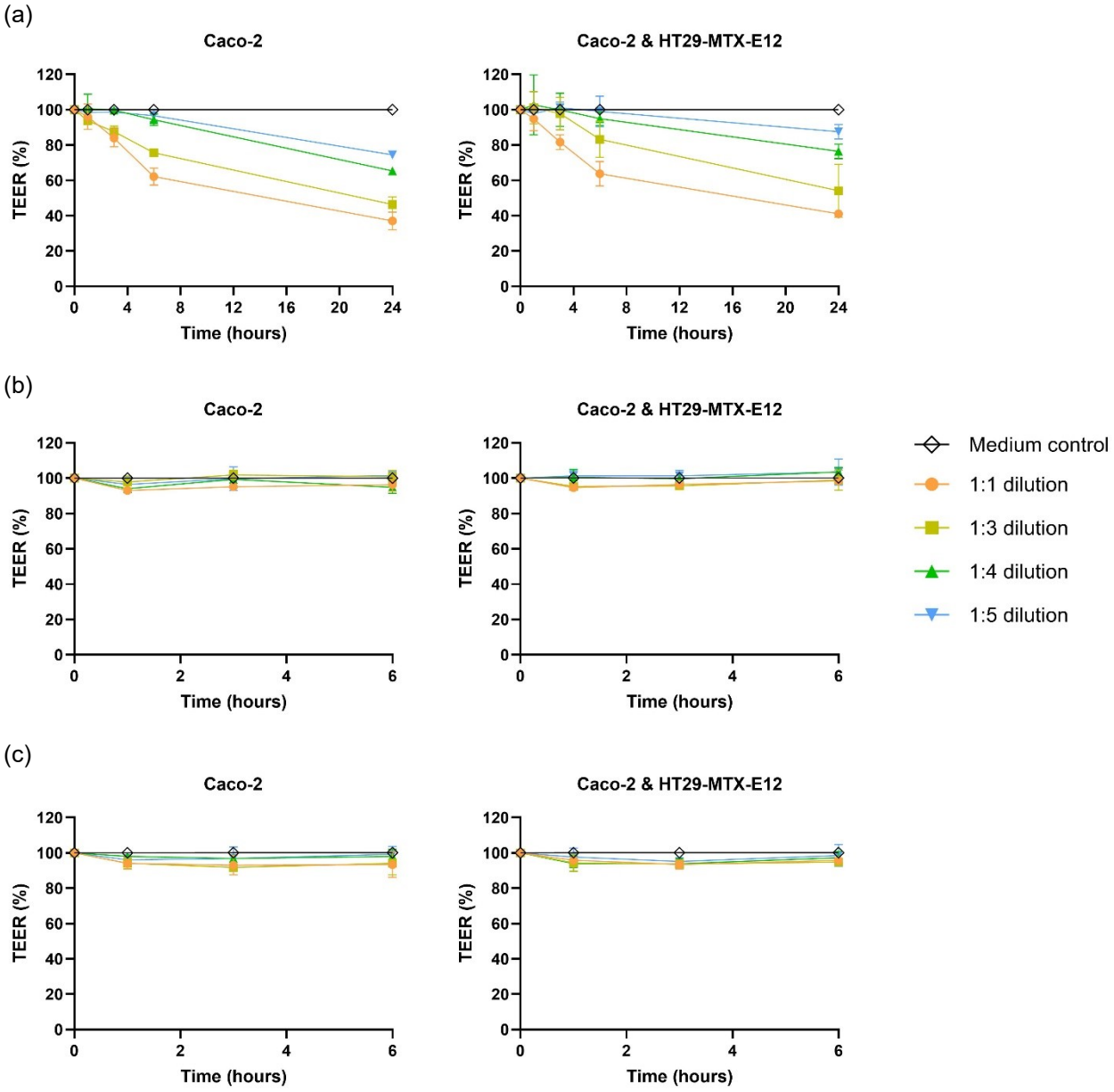


Figure S6: Representative images of mucus staining by Alcian Blue followed by PAS staining. Caco-2 and Caco-2/HT29-MTX-E12 cells (90:10) were co-cultured on Transwell™ inserts for 21 days, and stained with Alcian Blue (staining of acidic muco-substances as light blue) and PAS staining (staining of neutral muco-substances as deep red magenta). Cells that contain both neutral and acidic mucins will stain varying shades of purple due to the combination of binding of alcian blue and the reactivity with Schiff reagent (Suvarna et al., 2018). Bars, 100µm. A-B: Inserts fixated with Carnoy's solution before Alcian Blue/PAS staining; Carnoy's solution preserves well the neutral mucins within the monolayers. C-D: Inserts fixated with ethanol before Alcian Blue/PAS staining; Ethanol removes part of the neutral mucins, allowing staining of acidic mucins underneath.


(a)
Caco-2
Caco-2 & HT29-MTX-E12
TEER (%)
Time (hours)
(b)
Caco-2
Caco-2 & HT29-MTX-E12
TEER (%)
Time (hours)
(c)
Caco-2
Caco-2 & HT29-MTX-E12
TEER (%)
Time (hours)
Medium control
1:1 dilution
1:3 dilution
1:4 dilution
1:5 dilution

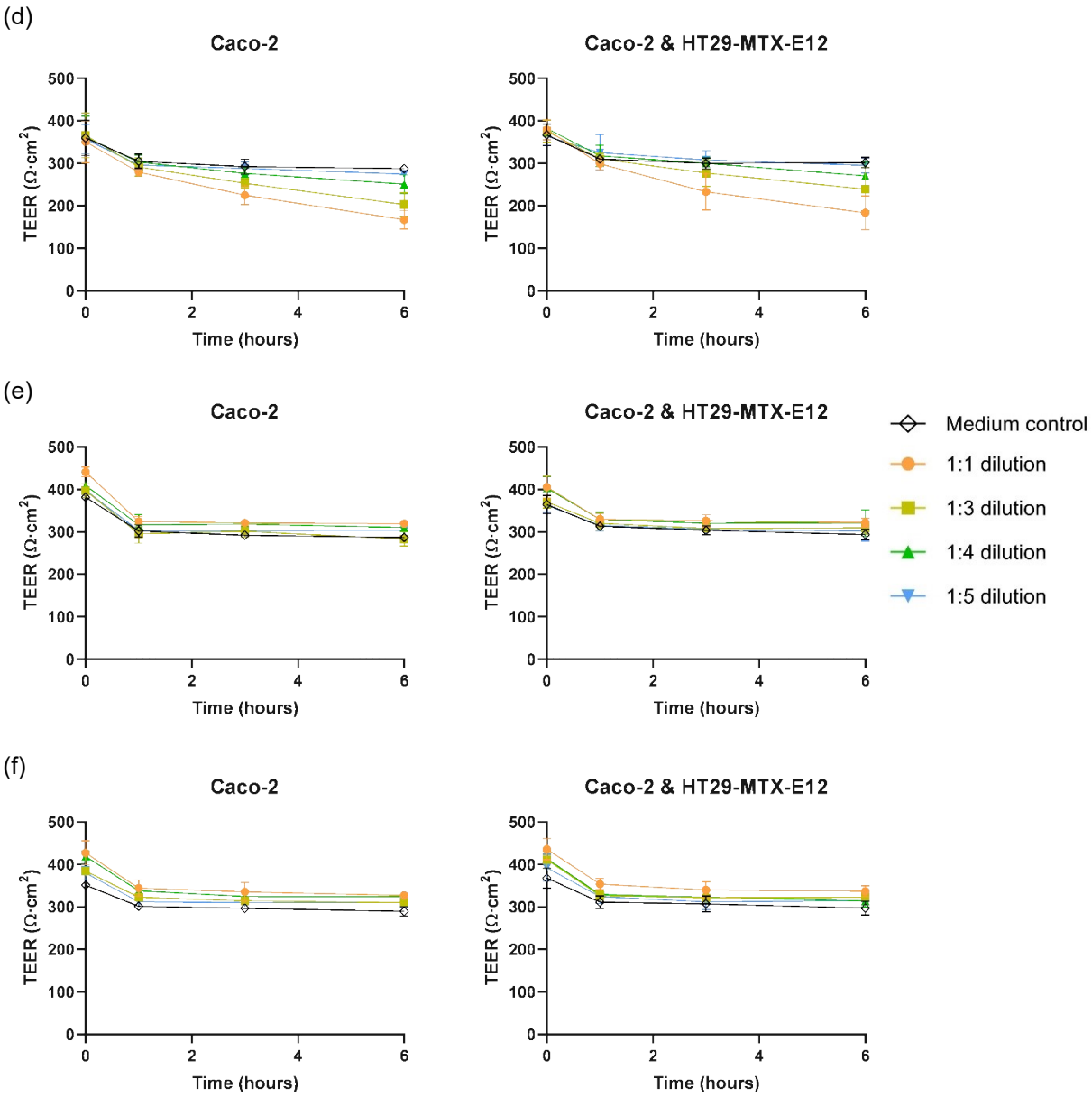


Figure S7: Transepithelial electrical resistance (TEER) values after intestinal digests, diluted at ratios from 1:1 to 1:5 in DMEM (without FBS), were applied to the apical side of the 21-days differentiated Caco-2 and Caco-2/HT29-MTX-E12 monolayers (n=3). Relative TEER values of (a) Not filtered control digest, (b) Filtered control digest, (c) Filtered unheated (UH) infant formula model system, and absolute TEER values of (d) Not filtered control digest, (e) Filtered control digest, (f) Filtered unheated (UH) infant formula model system. All samples were digested by use of an *in vitro* infant digestion model before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium control were Caco-2 and HT29-MTX-E12 cells exposed to DMEM. Error bars represent standard deviation.

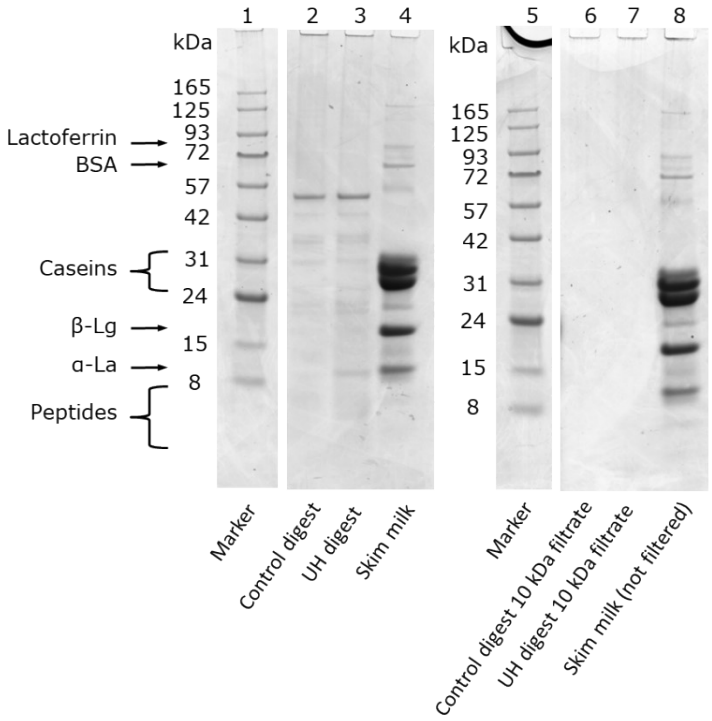


Figure S8: Reducing SDS-PAGE of intestinal digests before and after filtrationn of the digests by centrifugal filtration with a 10 kDa molecular weight cut off. An infant formula model system remained unheated (UH) and was digested by use of *an in vitro* infant digestion model. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Undigested, not filterated raw skim milk was measured as a control. Lane 1: marker, lane 2: control digest (not filtered), lane 3: UH digest (not filted), lane 4: skim milk (undigested, not filtered), lane 5: marker, lane 6: 10 kDa filtrate of control digest, lane 7: 10 kDa filtrate of UH digest, lane 8: skim milk (undigested, not filtered).

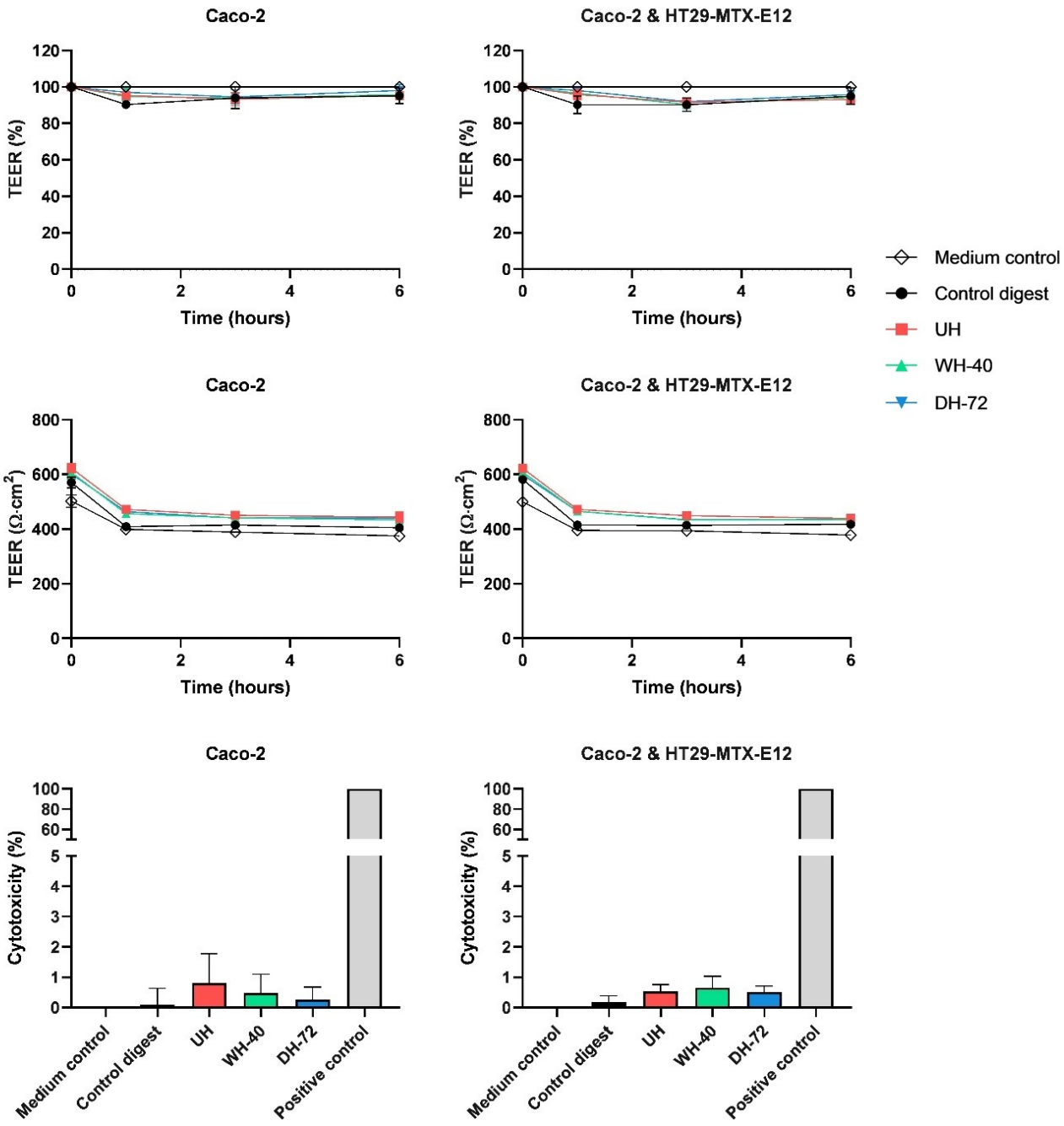
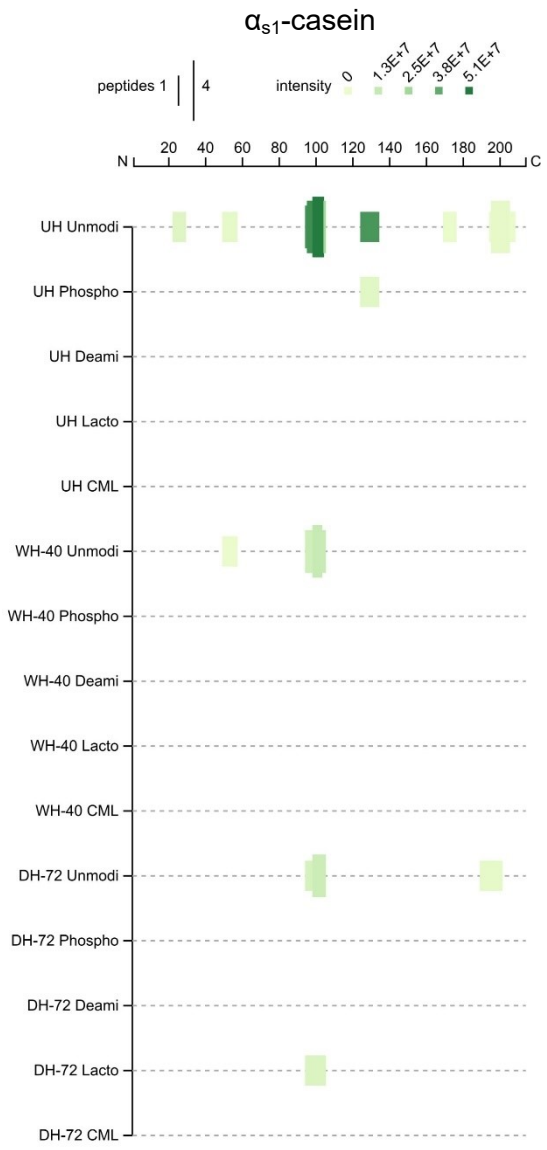


Figure S9: Relative and absolute transepithelial electrical resistance (TEER) values after 0-6h, and cytotoxicity effects 6h after filtered intestinal digests were applied to the apical side of the 21-days differentiated Caco-2 and Caco-2/HT29-MTX-E12 monolayers (n=3). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells for 0-6h (1:1 diluted in DMEM). Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Cytotoxicity was meausured with the LDH assay. Medium control was Caco-2 and HT29-MTX-E12 cells exposed to DMEM, and was set to 0%. The LDH positive control Triton X100 releases the maximum amount of LDH present within the cell monolayers, and was set at 100%. Error bars represent standard deviation.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UH Lacto
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WH-40 Phospho
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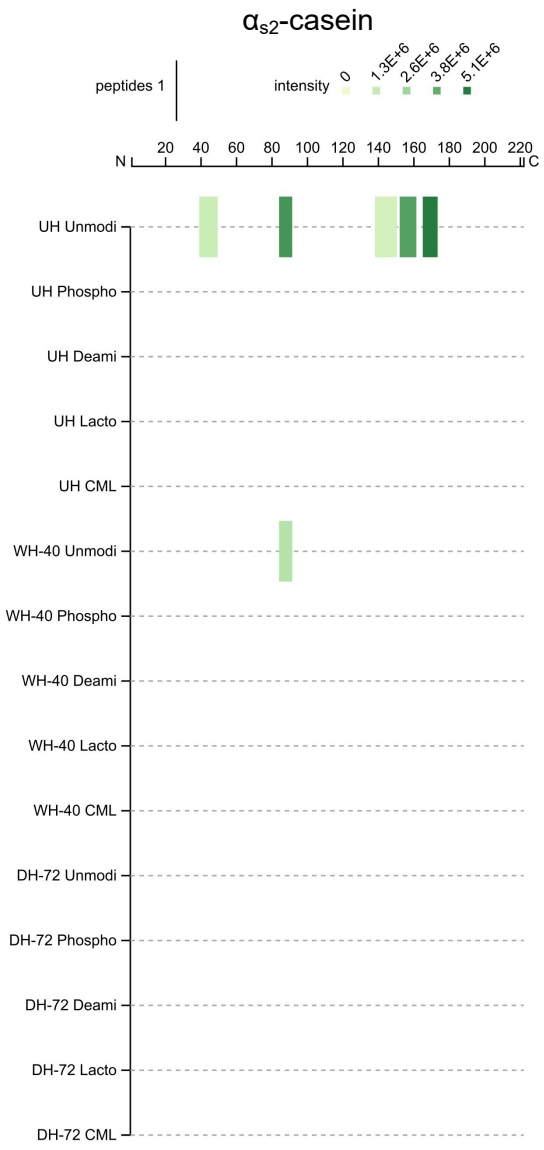
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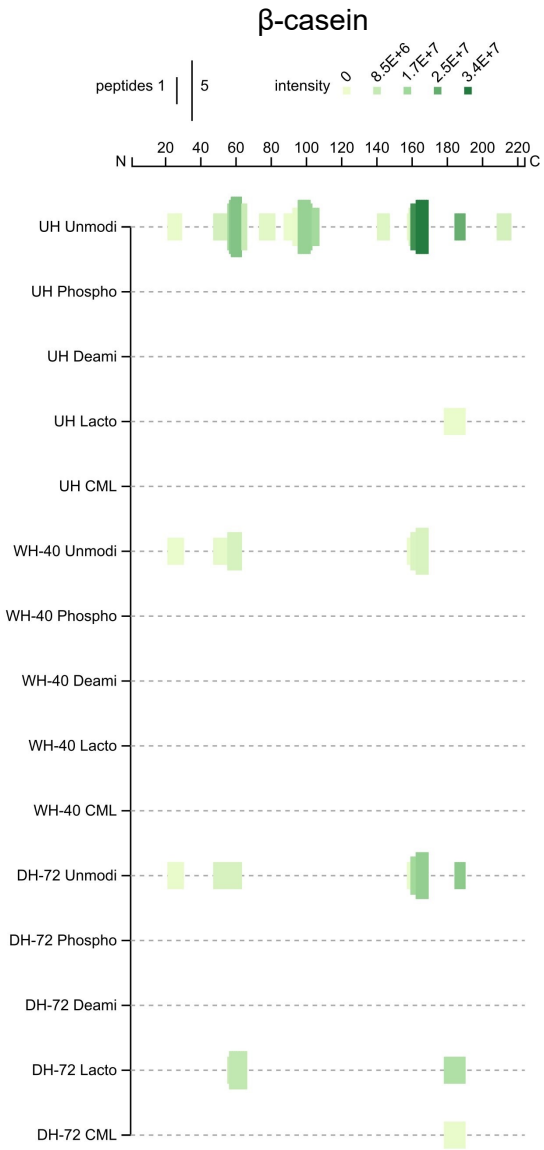
DH-72 Lacto
DH-72 CML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intensity
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UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
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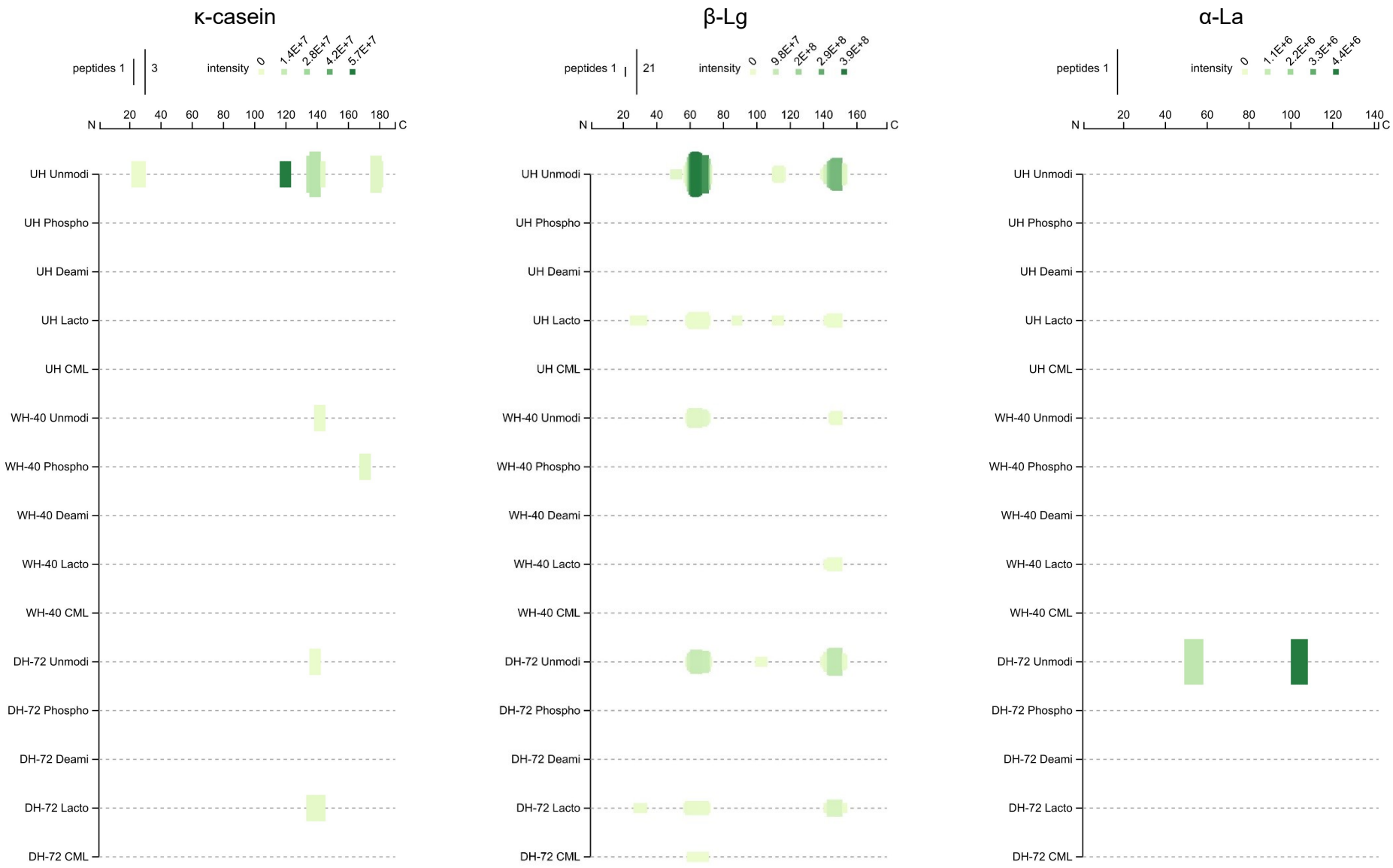
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UH CML
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WH-40 Deami
WH-40 Lacto
WH-40 CML
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DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
β-Lg
peptides 1
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intensity
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2E+8
2.9E+8
3.9E+8
N
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C
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WH-40 Lacto
WH-40 CML
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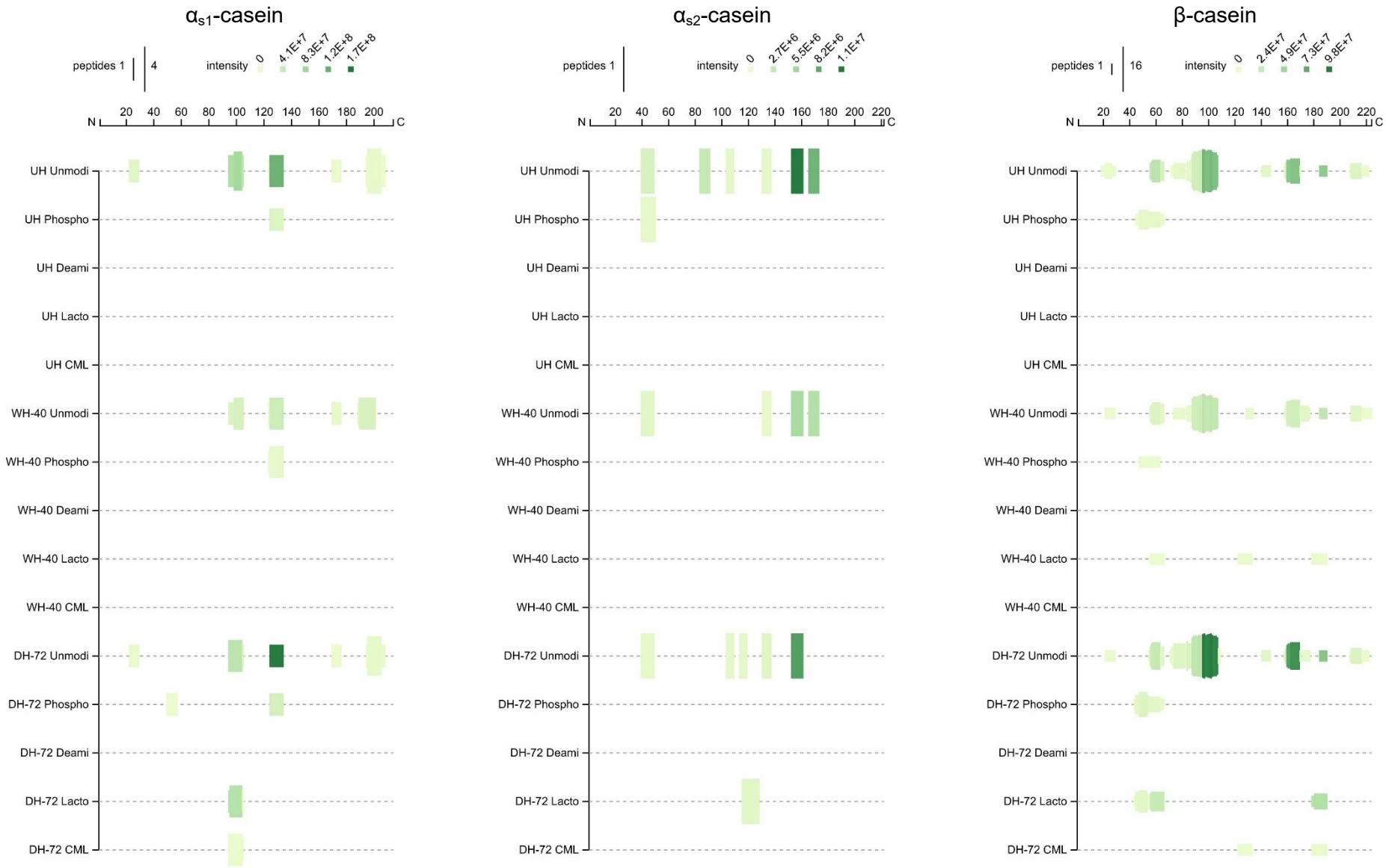
DH-72 Deami
DH-72 Lacto
DH-72 CML

Figure S10: Peptide alignment for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-lactoglobulin (β-Lg), and α-lactalbumin (α-La) detected with LC-MS/MS at the basolateral side after transport across a 21-days differentiated Caco-2 monolayer. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 cells for 6h. Peptide alignments are shown separately for unmodified (Unmodi), phosphorylated (Phospho), deamidation (Deami) modified, lactosylated (Lacto) and carboxymethyllysine (CML) modified peptides. The color of the bars represents the sum of peptide intensities that overlap at this position, and the height of the bars represents the number of peptides (peptide count) that overlap at this position. The amino acid position is given including the signal peptides of 15, 15, 15, 21, 16, and 19 amino acids for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-Lg, and α-La, respectively.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peptides 1
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intensity
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4.1E+7
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1.2E+8
1.7E+8
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C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
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DH-72 Deami
DH-72 Lacto
DH-72 CML
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intensity
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2.7E+6
5.5E+6
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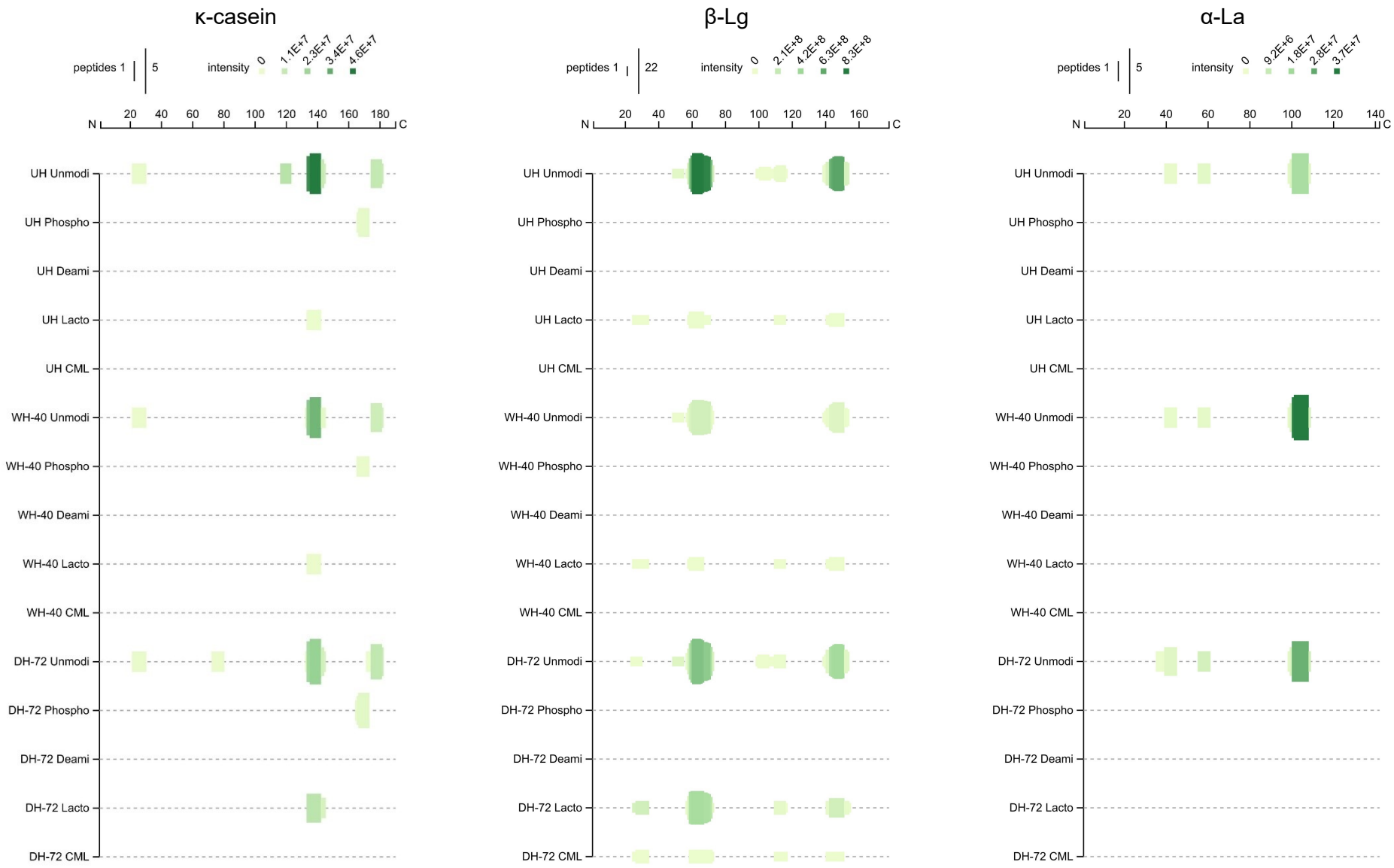
DH-72 Deami
DH-72 Lacto
DH-72 CML
β-casein
peptides 1
16
intensity
0
2.4E+7
4.9E+7
7.3E+7
9.8E+7
N
20 40 60 80 100 120 140 160 180 200 220
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML


κ-casein
peptides 1
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intensity
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3.4E+7
4.6E+7
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40
60
80
100
120
140
160
180
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
β-Lg
peptides 1
22
intensity
0
2.1E+8
4.2E+8
6.3E+8
8.3E+8
N
20
40
60
80
100
120
140
160
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML
α-La
peptides 1
5
intensity
0
9.2E+6
1.8E+7
2.8E+7
3.7E+7
N
20
40
60
80
100
120
140
C
UH Unmodi
UH Phospho
UH Deami
UH Lacto
UH CML
WH-40 Unmodi
WH-40 Phospho
WH-40 Deami
WH-40 Lacto
WH-40 CML
DH-72 Unmodi
DH-72 Phospho
DH-72 Deami
DH-72 Lacto
DH-72 CML

Figure S11: Peptide alignment for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-lactoglobulin (β-Lg), and α-lactalbumin (α-La) detected with LC-MS/MS at the basolateral side after transport across a 21-days differentiated Caco-2/HT29-MTX-E12 (90/10) monolayer. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells for 6h. Peptide alignments are shown separately for unmodified (Unmodi), phosphorylated (Phospho), deamidation (Deami) modified, lactosylated (Lacto), and carboxymethyllysine (CML) modified peptides. The color of the bars represents the sum of peptide intensities that overlap at this position, and the height of the bars represents the number of peptides (peptide count) that overlap at this position. The amino acid position is given including the signal peptides of 15, 15, 15, 21, 16, and 19 amino acids for α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, β-Lg, and α-La, respectively.

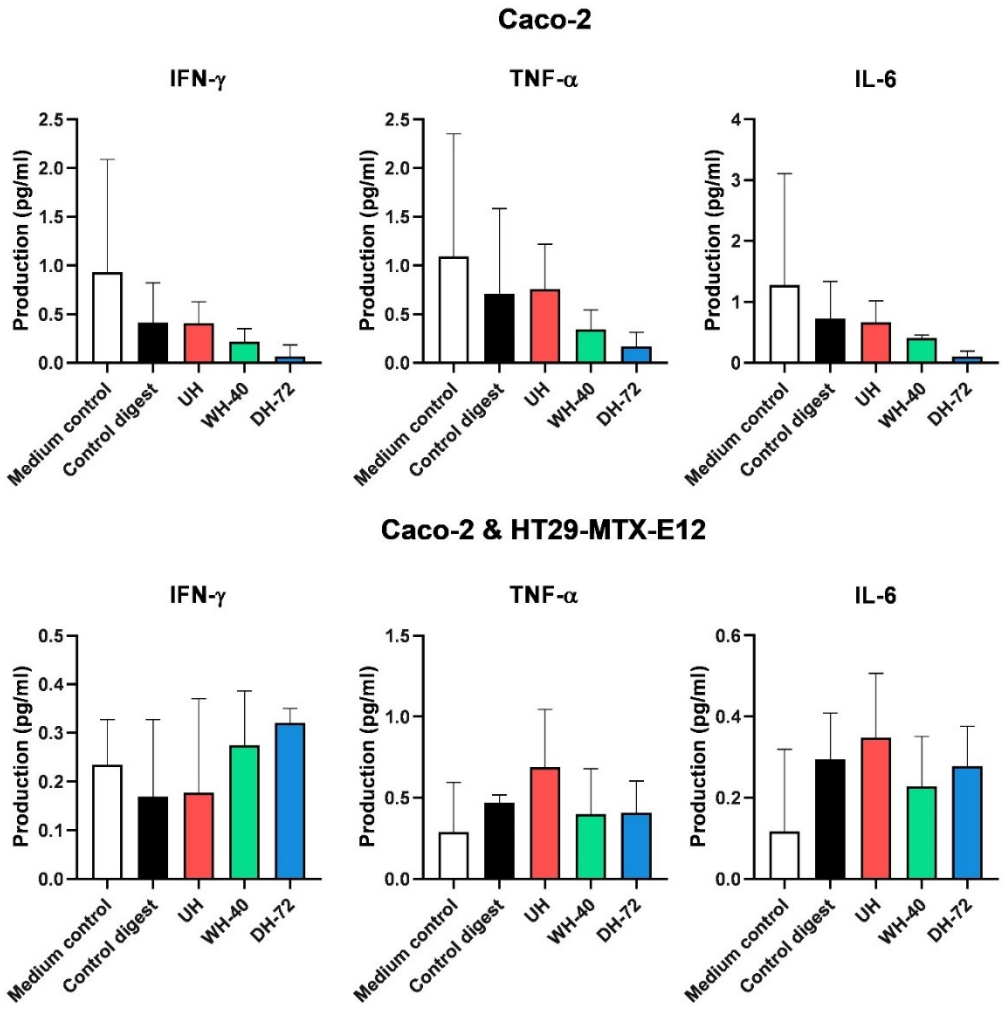

Figure S12: IFN-γ, TNF-α and IL-6 concentrations measured with a LEGENDplex assay at the basolateral side of a 21-days differentiated Caco-2 monolayer or a Caco-2/HT29-MTX-E12 (90/10) monolayer after stimulation with the filtered intestinal digests for 6h (n=3). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and Caco-2/HT29-MTX-E12 cells for 6h. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium controls were Caco-2 and Caco-2/HT29-MTX-E12 cells exposed to DMEM. Detection limits of IFN-γ, TNF-α, and IL-6 measured with the LEGENDplex assay were 0.25, 0.32, and 0.29 pg/ml, respectively. Cytokine concentrations were measured in triplicate, and the average of the three replicates is shown. If one of the replicates was below the detection limit, a value of zero was assigned to that replicate, and the mean was calculated accordingly. No statistical differences were found between the medium control, control digest, and milk digest samples. Error bars represent standard deviation.

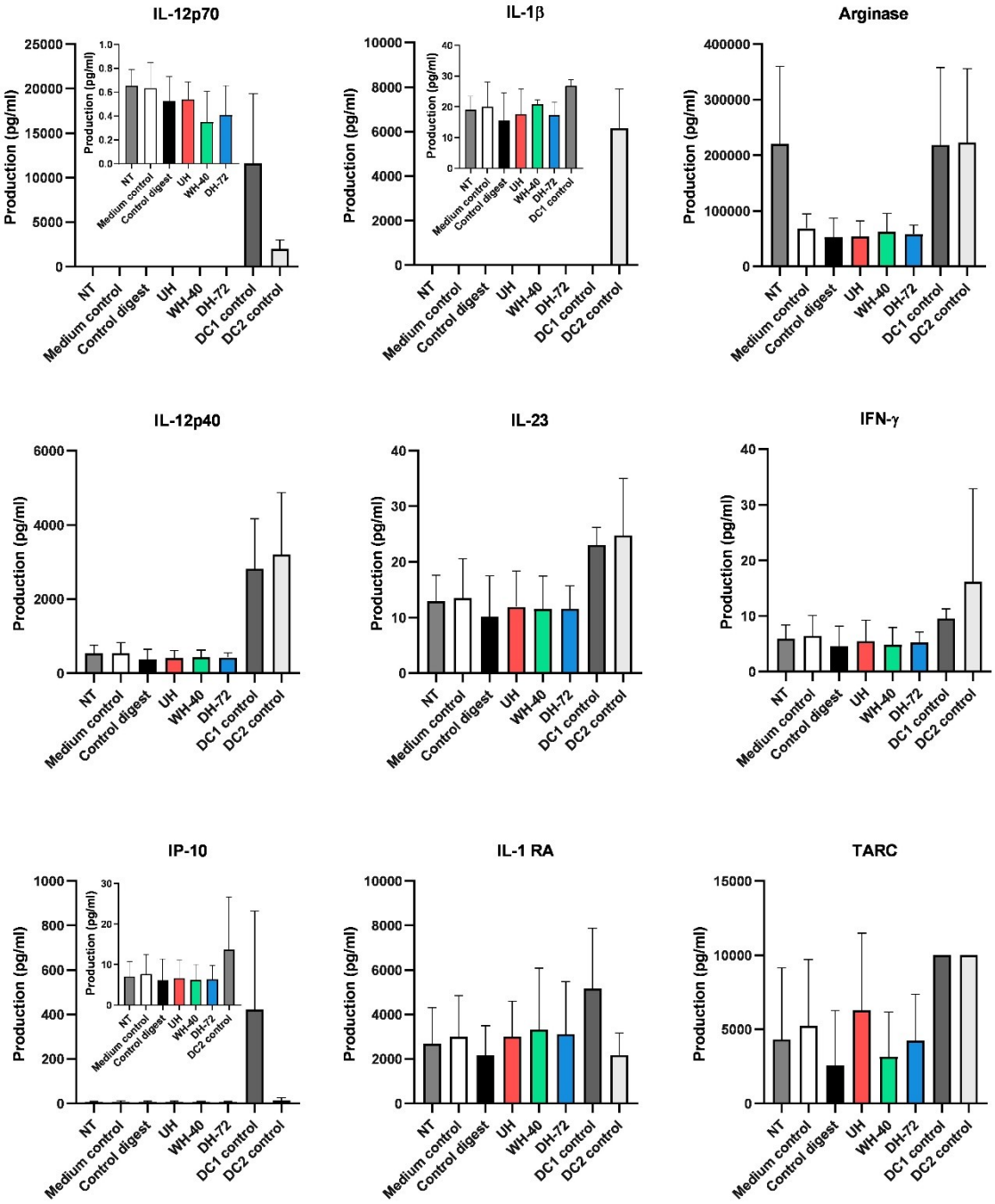

Figure S13: Cytokine concentrations after stimulating immature dendritic cells (iDCs) with medium from the basolateral side of a 21-days differentiated Caco-2 monolayer (n=5). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 cells for 6h. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium controls were Caco-2 cells exposed to DMEM. Non-treated (NT) iDCs, DC1 controls and DC2 controls were grown and stimulated in IMDM**.** The detection ranges of the cytokines measured with the LEGENDplex assay were: IL-12p70: 0.43 – 9000 pg/ml, IL-1β: 1.90 – 9000 pg/ml, arginase: 0.61 – 320000 pg/ml, IL-12p40: 13.3 – 45000 pg/ml, IL-23: 0.58 – 10000 pg/ml, IFN-γ: 0.98 – 9000 pg/ml, IP-10: 0.99 – 10000 pg/ml, IL-1RA: 64.6 – 400000 pg/ml, and TARC:

0.77 – 10000 pg/ml. Cytokine concentrations were measured in five replicates, and the average of the replicates is shown. If one of the replicates was below the detection limit, a value of zero was assigned to that replicate, and the mean was calculated accordingly. No statistical differences were found between NT, medium control, control digest, and milk digest samples. Error bars represent standard deviation.

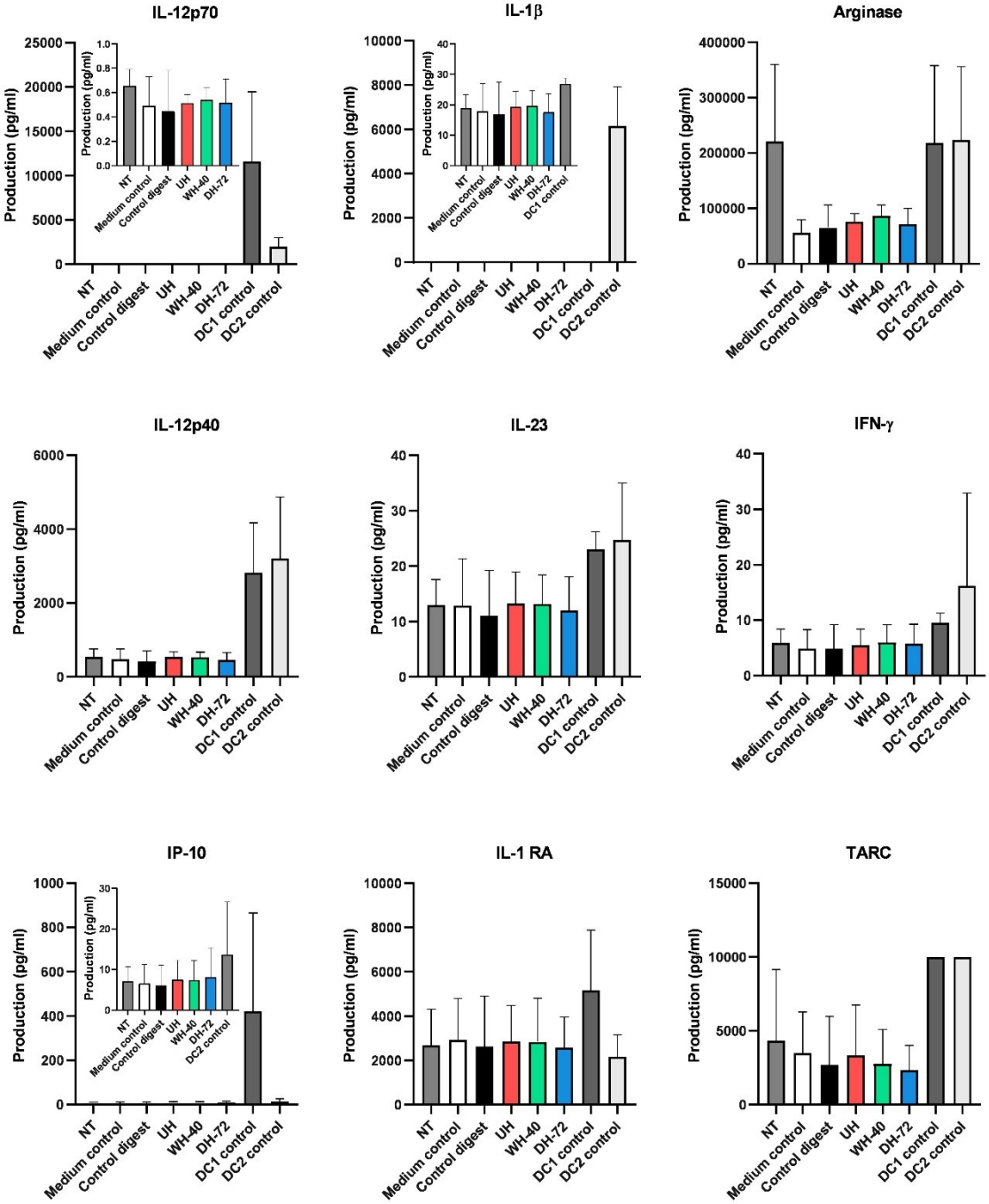

Figure S14: Cytokine concentrations after stimulating immature dendritic cells (iDCs) with medium from the basolateral side of a 21-days differentiated Caco-2/HT29-MTX-E12 (90/10) monolayer (n=5). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2/HT29-MTX-

E12 cells for 6h. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest. Medium controls were Caco-2/HT29-MTX-E12 cells exposed to DMEM. Non-treated (NT) iDCs, DC1 controls and DC2 controls were grown and stimulated in IMDM. The detection ranges of the cytokines measured with the LEGENDplex assay were: IL-12p70: 0.43 – 9000 pg/ml, IL-1β: 1.90 – 9000 pg/ml, arginase: 0.61 – 320000 pg/ml, IL-12p40: 13.3 – 45000 pg/ml, IL-23: 0.58 – 10000 pg/ml, IFN-γ: 0.98 – 9000 pg/ml, IP-10: 0.99 – 10000 pg/ml, IL-1RA: 64.6 – 400000 pg/ml, and TARC: 0.77 – 10000 pg/ml. Cytokine concentrations were measured in five replicates, and the average of the replicates is shown. If one of the replicates was below the detection limit, a value of zero was assigned to that replicate, and the mean was calculated accordingly. No statistical differences were found between NT, medium control, control digest, and milk digest samples. Error bars represent standard deviation.

Table S1: qPCR forward and reverse primers details used in mucins gene expression analysis. MUC: mucin; CF3A1: Splicing factor 3 subunit 1; HPRT1: Hypoxanthine Phosphoribosyl Transferase 1; hCyclo: human Cyclophilin A (PPIA); RPS18: Ribosomal protein S18; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. For MUC2, MUC6, MUC12 and MUC15 the primer efficiency could not be determined (ND), as the genes were barely expressed in our models.

Gene	**Forward primer 5'-3'**	**Reverse primer 5'-3'**	**[Primer]**	**Efficiency**	**Reference**
MUC1	TGCCGCCGAAAGAACTACG	TGGGGTACTCGCTCATAGGAT	3.2 µM	102.7%	PrimerBank ID 324120973c1
MUC2	AGGATGACACCATCTACCTCAC	CATCGCTCTTCTCAATGAGCA	3.2 µM	ND	PrimerBank ID 116284391c2
MUC3A	CCGGACCTCAATGACAACACT	ACCACGATGCTGCCATTCCT	3.2 µM	99.4%	Pan et al.,2013
MUC3B	TGACGCTCAGCAAAACCGATAAC	AACAGGCTTCAGGACCAAGACAGC	3.2 µM	104.2%	George et al.,2018
MUC5A	CAGCACAACCCCTGTTTCAAA	GCGCACAGAGGATGACAGT	3.2 µM	94.1%	PrimerBank ID 3334747a1
MUC6	TGGTGAACTCGTGGAAGGA	TGGCAGGTGGCAAAGGT	3.2 µM	ND	Shi & Xi, 2021
MUC12	CCAGTTCAAGCGACCCTTTTA	CGCTGTGGGATACTGTTGATT	3.2 µM	ND	PrimerBank ID 256600256c2
MUC13	GATCCCTGTGCAGATAATTCGTT	ACTATGCAAGTCTTGATAGGCCA	3.2 µM	101.0%	PrimerBank ID 308736984c2
MUC15	TATTCACTTCTATCGGGGAGCC	GGGAATGACTCGCCTTGAGAT	3.2 µM	ND	PrimerBank ID 205360922c1
MUC17	TCTCAGCACGTTAGGACAGGT	TCGAGGTCATCTCAGGGTTGG	3.2 µM	82.6%	PrimerBank ID 91982771c1
SF3A1	GGAGGATTCTGCACCTTCTAA	GCGGTAGTAGGCATGGTAA	3.2 µM	96.6%	Szabo et al., 2004
HPRT1	CCTGGCGTCGTGATTAGTGAT	AGACGTTCAGTCCTGTCCATAA	3.2 µM	97.5%	Primerbank 164518913c1
RPS18	ATCACCATTATGCAGAATCCACG	GACCTGGCTGTATTTTCCATCC	3.2 µM	92.0%	PrimerBank ID 14165467c2
hCyclo	CCCACCGTGTTCTTCGACAT	TCTTTGGGACCTTGTCTGCAA	1.6 µM	89.2%	Own design
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG	1.6 µM	89.0%	Vandesompele et al., 2002

Table S2: HLA-II epitopes predicited in the filtered intestinal digests by use of the IEDB MHC Class II Binding Prediction tool. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model. Amino acid (AA) position provides the position of the peptide containing an HLA epitope in the protein without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form.

Protein of origin	Peptide	AA position	HLA gene	Intensity UH	Intensity WH-40	Intensity DH-72	Modification
α_{s1}-casein	FFVAPFPEVFG	23-33	HLA-DP, HLA-DQ, HLA-DR	9.14E+06	6.57E+08	1.08E+07	Unmodified
	KYKVPQLEIVPNSAEER	103-119	HLA-DR		1.13E+08		Phosphorylation
	YKVPQLEIVPNSAEE	104-118	HLA-DR	1.24E+07	1.96E+08		Phosphorylation
	YKVPQLEIVPNSAEER	104-119	HLA-DR	1.02E+08	1.35E+09	2.78E+07	Phosphorylation
	KVPQLEIVPNSAEE	105-118	HLA-DR		2.91E+08		Phosphorylation
	KVPQLEIVPNSAEER	105-119	HLA-DR	3.48E+08	2.78E+09	7.26E+07	Phosphorylation, Deamidation
	VPQLEIVPNSAEER	106-119	HLA-DR	2.97E+08	1.55E+09	2.11E+08	Phosphorylation, Deamidation
	PQLEIVPNSAEER	107-119	HLA-DR		1.81E+06		Phosphorylation
	QLEIVPNSAEER	108-119	HLA-DR	1.59E+07	1.44E+07	1.24E+07	Phosphorylation
	LEIVPNSAEER	109-119	HLA-DR	1.29E+09	4.50E+09	7.07E+08	Phosphorylation, Deamidation
	EGIHAQQKEPM	125-135	HLA-DR	2.67E+07	2.48E+07	5.37E+07	Unmodified, Deamidation
	EGIHAQQKEPMIGV	125-138	HLA-DR		7.44E+06	7.51E+06	Unmodified
	EPMIGVNQELA	133-143	HLA-DQ	4.10E+07	1.10E+08	8.49E+07	Unmodified
	TDAPSFSDIPNPIGSEN	174-190	HLA-DR	1.52E+07	4.29E+07	4.11E+07	Unmodified
α_{s2}-casein	NMAINPSKENL	25-35	HLA-DR	6.37E+08	2.68E+08	3.67E+08	Unmodified, Phosphorylation, Lactosylation
	KITVDDKHYQK	70-80	HLA-DR		3.53E+07		Unmodified
	YQGPIVLNPWDQV	100-112	HLA-DR	4.30E+07	1.10E+07	2.60E+08	Unmodified
	YQGPIVLNPWDQVK	100-113	HLA-DR	5.29E+06		7.59E+08	Lactosylation
	QGPIVLNPWDQV	101-112	HLA-DR, HLA-DP	6.50E+07	1.09E+08	1.31E+08	Unmodified
	QGPIVLNPWDQVK	101-113	HLA-DR, HLA-DP	2.93E+06		2.31E+08	Lactosylation
	TKLTEEEKNRL	151-161	HLA-DR		1.21E+07		Unmodified
	TKLTEEEKNRLN	151-162	HLA-DR	3.32E+07	2.56E+07		Unmodified
β-casein	EELNVPGEIVES	4-15	HLA-DQ	6.18E+07	3.06E+07	2.11E+07	Unmodified
	EELNVPGEIVESL	4-16	HLA-DQ	2.21E+07	2.46E+07	8.93E+06	Phosphorylation
	LNVPGEIVESL	6-16	HLA-DQ	5.54E+06	5.87E+06	5.69E+06	Phosphorylation

	KIEKFQSEEQQ	29-39	HLA-DR, HLA-DQ			1.21E+07	Phosphorylation, CML
	TPVVVPPFLQP	80-90	HLA-DP	5.35E+07	2.01E+07	9.39E+06	Unmodified
	TPVVVPPFLQPEV	80-92	HLA-DP	1.14E+08	1.04E+08	6.29E+07	Unmodified
	PVVVPPFLQPE	81-91	HLA-DP	2.82E+08	9.53E+07	1.48E+08	Unmodified
	SLSQSKVLPVPQ	164-175	HLA-DR, HLA-DQ	3.20E+06	2.30E+08	6.89E+09	Lactosylation, CML
	LSQSKVLPVPQ	165-175	HLA-DQ			9.71E+07	Lactosylation
κ-casein	ALINNQFLPYP	49-59	HLA-DP, HLA-DR, HLA-DQ	2.55E+05	1.32E+07		Unmodified
	TIASGEPTSTPT	124-135	HLA-DQ	2.16E+07	1.34E+07	1.69E+07	Unmodified, Phosphorylation
	IASGEPTSTPT	125-135	HLA-DQ	1.48E+07	8.21E+06		Unmodified
	IASGEPTSTPTTE	125-137	HLA-DQ	5.18E+07	1.21E+07	4.64E+07	Unmodified
	IASGEPTSTPTTEA	125-138	HLA-DQ	4.12E+07	1.23E+07	3.74E+07	Unmodified
β-Lg	IIAEKTKIPAVF	71-82	HLA-DR	9.09E+06			Unmodified
	KIDALNENKVL	83-93	HLA-DR	4.85E+08	1.58E+07	1.03E+08	Unmodified, CML
	KIDALNENKVLV	83-94	HLA-DR	8.74E+07		3.48E+07	Unmodified, CML
	VLVLDTDYKKY	92-102	HLA-DR	4.73E+07	3.80E+06		Unmodified
	TPEVDDEALEKF	125-136	HLA-DQ	1.95E+09	1.11E+08	1.39E+09	Unmodified, Lactosylation, CML
	LSFNPTQLEEQ	149-159	HLA-DQ	4.47E+07		7.06E+07	Unmodified
α-La	GYGGVSLPEWV	17-27	HLA-DQ	5.18E+07	6.86E+07	7.95E+07	Unmodified
	IVQNNDSTEYG	41-51	HLA-DR	3.21E+07	1.58E+08	2.55E+08	Unmodified, Deamidation
Osteopontin	DKNKHSNLIESQ	207-218	HLA-DQ	8.50E+06	6.65E+06		Phosphorylation
Desmoplakin	LQKYQAECSQFK	1072-1083	HLA-DR	3.34E+08	1.30E+07	5.26E+07	Phosphorylation, CML
GRIP and coiled-coil domain containing 2	IDQLKLKLQDTQNS	1358-1371	HLA-DR	5.98E+08	6.92E+07	4.32E+08	Deamidation, CML
Inositol 1,4,5-trisphosphate receptor	IQKSFYNLMTS	1761-1771	HLA-DP, HLA-DQ, HLA-DR	1.71E+08	2.91E+07	4.26E+07	Phosphorylation
Nucleobindin-1	ILHDINSDGVLDEQ	247-260	HLA-DQ, HLA-DR		1.38E+07	1.99E+07	Unmodified
Polymeric immunoglobulin receptor	KVVQGEPSLKVPK	455-467	HLA-DP, HLA-DR	3.25E+07			Unmodified
TSPO associated protein 1	WQSWTPGRGGDA	24-35	HLA-DQ	6.66E+07	1.61E+07		Deamidation, Phosphorylation
Count				**42**	**43**	**37**	

Total Intensity		**7.54E+09**	**1.32E+10**	**1.28E+10**

Table S3: Linear IgE epitopes predicted in the filtered intestinal digests. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model. Amino acid (AA) position provides the position of the peptide containing a linear IgE epitope in the protein without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form.

Protein of origin	Peptide	AA position	IgE epitope position	Intensity UH	Intensity WH-40	Intensity DH-72	Modification
α_{s1}-casein	HQGLPQEVLNENL	8-20	6-20		6.39E+06		Unmodified
	KYKVPQLEIVPNSAEER	103-119	109-120		1.13E+08		Phosphorylation
	YKVPQLEIVPNSAEE	104-118	109-120	1.24E+07	1.96E+08		Phosphorylation
	YKVPQLEIVPNSAEER	104-119	109-120	1.02E+08	1.35E+09	2.78E+07	Phosphorylation
	KVPQLEIVPNSAEE	105-118	109-120		2.91E+08		Phosphorylation
	KVPQLEIVPNSAEER	105-119	109-120	3.48E+08	2.78E+09	7.26E+07	Phosphorylation, Deamidation
	VPQLEIVPNSAEER	106-119	109-120	2.97E+08	1.55E+09	2.11E+08	Phosphorylation, Deamidation
	PQLEIVPNSAEER	107-119	109-120		1.81E+06		Phosphorylation
	QLEIVPNSAEER	108-119	109-120	1.59E+07	1.44E+07	1.24E+07	Phosphorylation
	LEIVPNSAEE	109-118	109-120	1.05E+08	5.87E+08	5.71E+07	Phosphorylation
	LEIVPNSAEER	109-119	109-120	1.29E+09	4.50E+09	7.07E+08	Phosphorylation, Deamidation
	EIVPNSAEER	110-119	109-120	7.23E+09	1.65E+09	6.66E+09	Phosphorylation, Deamidation
	LHSMKEGIHAQ	120-130	122-132		5.89E+07		Unmodified
	SMKEGIHAQ	122-130	122-132		1.26E+07		Unmodified
	EGIHAQQKEPMIGV	125-138	126-140		7.44E+06	7.51E+06	Unmodified
	EGIHAQQKEPMIGVNQEL	125-142	126-140		1.09E+07		Unmodified
	EGIHAQQKEPMIGVNQELA	125-143	126-140		9.74E+07		Unmodified
	AQQKEPMIGVNQEL	129-142	126-140	7.58E+06	5.82E+06	1.16E+07	Unmodified
	AQQKEPMIGVNQELA	129-143	126-140	1.34E+07	1.86E+07	1.59E+07	Unmodified
	QYTDAPSFSDIPNPI	172-186	171-185		1.07E+09		Unmodified
	QYTDAPSFSDIPNPIGSENSEK	172-193	171-185; 173-194		1.59E+07	7.01E+06	Unmodified
	YTDAPSFSDIPNPI	173-186	171-185	2.95E+08	4.47E+07	2.99E+08	Unmodified
	TDAPSFSDIPNPI	174-186	171-185	2.95E+09	7.23E+06	1.27E+07	Unmodified, Phosphorylation
	TDAPSFSDIPNPIGSEN	174-190	171-185	1.52E+07	4.29E+07	4.11E+07	Unmodified

	TDAPSFSDIPNPIGSENSEK	174-193	171-185; 173-194	1.76E+07	1.15E+08	5.57E+07	Unmodified
	TDAPSFSDIPNPIGSENSEKT	174-194	171-185; 173-194	5.19E+06	3.18E+07	1.02E+07	Unmodified
α_{s2}-casein	YQGPIVLNPWDQV	100-112	105-114	4.30E+07	1.10E+07	2.60E+08	Unmodified
	YQGPIVLNPWDQVK	100-113	105-114	5.29E+06		7.59E+08	Lactosylation
	QGPIVLNPWDQV	101-112	105-114	6.50E+07	1.09E+08	1.31E+08	Unmodified
	QGPIVLNPWDQVK	101-113	105-114	2.93E+06		2.31E+08	Lactosylation
β-casein	EELNVPGEIVESL	4-16	1-16	2.21E+07	2.46E+07	8.93E+06	Phosphorylation
	INKKIEKFQSEEQQQTEDELQDK	26-48	25-50			4.31E+07	Phosphorylation, Lactosylation
	KIEKFQSEEQQQTEDELQDKIHP	29-51	25-50	5.51E+06		9.03E+07	Phosphorylation, Lactosylation
	IEKFQSEEQQQTEDELQDKIHP	30-51	25-50	5.99E+07	3.18E+07	2.03E+08	Phosphorylation, Lactosylation
	FQSEEQQQTEDELQDKIHPF	33-52	45-54	6.00E+06	3.83E+07		Phosphorylation
	QTEDELQDKIHPF	40-52	45-54		8.28E+06		Unmodified
	QTEDELQDKIHPFA	40-53	45-54			6.53E+07	Lactosylation
	TEDELQDKIHPF	41-52	45-54	2.22E+07	5.85E+07	1.94E+07	Unmodified
	TEDELQDKIHPFA	41-53	45-54		1.65E+07	4.61E+07	Unmodified, Lactosylation
	QDKIHPFA	46-53	45-54	2.91E+08	1.48E+08	1.85E+08	Unmodified
	QSLVYPFPGPIPN	56-68	55-70	9.38E+08	1.04E+09	1.23E+09	Unmodified
	QSLVYPFPGPIPNS	56-69	55-70	2.50E+07	2.61E+07	4.70E+07	Unmodified
	SLVYPFPGPIPNS	57-69	55-70	5.53E+07	1.01E+08	6.95E+07	Unmodified
	NSLPQNIPPLTQTPVVVPPFLQPEV	68-92	83-92	1.73E+07	8.40E+06	6.44E+07	Unmodified
	SLPQNIPPLTQTPVVVPPFLQP	69-90	83-92	9.03E+06	5.56E+06	1.61E+07	Unmodified
	SLPQNIPPLTQTPVVVPPFLQPE	69-91	83-92	4.14E+07	4.43E+07	3.83E+07	Unmodified
	SLPQNIPPLTQTPVVVPPFLQPEV	69-92	83-92	1.05E+09	7.36E+08	1.71E+09	Unmodified, Deamidation
	LPQNIPPLTQTPVVVPPFLQPEV	70-92	83-92	3.67E+07	2.71E+07	6.88E+07	Unmodified
	QNIPPLTQTPVVVPPFLQPEV	72-92	83-92	7.60E+06	4.16E+06	1.21E+07	Unmodified
	NIPPLTQTPVVVPPFLQP	73-90	83-92	2.10E+07	1.63E+07	2.41E+07	Unmodified
	NIPPLTQTPVVVPPFLQPE	73-91	83-92	8.77E+07	7.92E+07	6.09E+07	Unmodified
	NIPPLTQTPVVVPPFLQPEV	73-92	83-92	1.45E+09	7.79E+08	1.22E+09	Unmodified
	NIPPLTQTPVVVPPFLQPEVMG	73-94	83-92	1.71E+07	1.31E+07	2.76E+07	Unmodified

	IPPLTQTPVVVPPFLQPEV	74-92	83-92	5.80E+07	5.18E+07	1.16E+08	Unmodified
	PPLTQTPVVVPPFLQPEV	75-92	83-92	8.31E+06	5.52E+06		Unmodified
	TQTPVVVPPFLQPEV	78-92	83-92	2.34E+08	2.73E+08	1.47E+08	Unmodified
	QTPVVVPPFLQP	79-90	83-92	7.30E+07	4.23E+07	2.50E+07	Unmodified
	QTPVVVPPFLQPE	79-91	83-92	2.06E+07	1.23E+07	2.03E+07	Unmodified
	QTPVVVPPFLQPEV	79-92	83-92	4.17E+08	2.45E+08	6.43E+08	Unmodified, Deamidation
	QTPVVVPPFLQPEVMG	79-94	83-92	7.39E+06	6.53E+06	1.61E+07	Unmodified
	TPVVVPPFLQP	80-90	83-92	5.35E+07	2.01E+07	9.39E+06	Unmodified
	TPVVVPPFLQPEV	80-92	83-92	1.14E+08	1.04E+08	6.29E+07	Unmodified
	PVVVPPFLQP	81-90	83-92	4.06E+07	1.39E+07	3.60E+07	Unmodified
	PVVVPPFLQPE	81-91	83-92	2.82E+08	9.53E+07	1.48E+08	Unmodified
	PVVVPPFLQPEV	81-92	83-92	3.35E+07	2.00E+09	5.17E+07	Unmodified, Deamidation
	PVVVPPFLQPEVM	81-93	83-92	3.98E+07	3.26E+06	1.26E+07	Unmodified
	PVVVPPFLQPEVMG	81-94	83-92	1.55E+08	3.72E+07	1.52E+08	Unmodified
	VVVPPFLQPEV	82-92	83-92	6.41E+07	3.72E+07	5.65E+07	Unmodified
	VVPPFLQPE	83-91	83-92	3.83E+08	4.72E+08	1.68E+08	Unmodified
	VVPPFLQPEV	83-92	83-92	7.23E+09	9.13E+09	5.90E+09	Unmodified, Deamidation
	VVPPFLQPEVMG	83-94	83-92	1.28E+08	1.57E+08	1.36E+08	Unmodified
	VPPFLQPEV	84-92	83-92	1.30E+07	1.01E+07	6.93E+06	Unmodified
	PPFLQPEV	85-92	83-92	2.55E+08	2.49E+08	2.19E+08	Unmodified
	HKEMPFPKYPVEP	106-118	107-120	3.07E+06	3.50E+06	3.28E+07	Lactosylation
	HKEMPFPKYPVEPF	106-119	107-120		2.40E+07	3.33E+07	Unmodified, Lactosylation
	EMPFPKYPVEPF	108-119	107-120	2.89E+06	1.90E+08	2.28E+09	Unmodified, Lactosylation
	WMHQPHQPLPPTVMFPPQS	143-161	149-164		6.31E+06		Unmodified
	MHQPHQPLPPTVMFPPQS	144-161	149-164	1.00E+08	7.57E+08		Unmodified
	MHQPHQPLPPTVMFPPQSV	144-162	149-164	1.96E+08	1.09E+09	3.68E+07	Unmodified
	HQPHQPLPPTVMFPPQS	145-161	149-164	2.29E+07	2.61E+08		Unmodified
	HQPHQPLPPTVMFPPQSV	145-162	149-164	1.03E+08	5.74E+08	8.22E+06	Unmodified
	QPHQPLPPTVMFPPQSV	146-162	149-164		3.52E+06		Unmodified
	QPLPPTVMFPPQS	149-161	149-164		5.99E+07	1.28E+06	Unmodified
	QPLPPTVMFPPQSV	149-162	149-164	1.85E+07	1.22E+08	2.83E+06	Unmodified

κ-casein	MAIPPKKNQDKTEIPTINT	106-124	111-126			1.72E+07	Lactosylation
	KNQDKTEIPTINT	112-124	111-126	8.11E+08	2.74E+08	3.78E+08	Unmodified, Lactosylation
β-Lg	LIVTQTMKGLDIQK	1-14	1-16			3.06E+06	CML
	VRTPEVDDEALEKFDK	123-138	121-140	2.72E+09	5.12E+07	1.46E+09	Unmodified, Lactosylation, CML
	VRTPEVDDEALEKFDKA	123-139	121-140	5.50E+07		2.24E+08	Unmodified, Lactosylation
	RTPEVDDEALEKFDKA	124-139	121-140	9.64E+06		4.21E+06	Unmodified
	TPEVDDEALEKFDKAL	125-140	121-140	2.34E+07		1.43E+09	Unmodified, Lactosylation, CML
	TPEVDDEALEKFDKALK	125-141	121-140	4.28E+07		1.28E+08	Unmodified, Lactosylation
α-La	KILDKVGI	94-101	93-102	4.55E+07	7.83E+07	2.37E+07	Unmodified
	KILDKVGIN	94-102	93-102	1.27E+08	2.05E+08	7.91E+07	Unmodified, CML
	ILDKVGIN	95-102	93-102	3.20E+08	4.92E+08	5.14E+08	Unmodified, CML
	ILDKVGINY	95-103	93-102		3.24E+07		Unmodified
Count				**73**	**84**	**77**	
Total Intensity				**3.04E+10**	**3.48E+10**	**2.91E+10**	

Table S4: Bioactive peptides identified in the filtered intestinal digests by use of the milk bioactive peptide database (MBPDB). An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model. Amino acid (AA) position provides the position of the peptide that was identical to a peptide in the MBPDB without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form.

Protein of origin	Peptide	AA position	Bioactive function	Intensity UH	Intensity WH-40	Intensity DH-72	Modification
α_{s1}-casein	FVAPFPEVFG	24-33	ACE-inhibitory	5.47E+08	3.49E+08	8.81E+08	Unmodified
	DIGSESTEDQAMEDIK	43-58	Promotes calcium uptake	3.31E+07	7.22E+06	8.13E+06	Phosphorylation
	YKVPQLEIVPNSAEER	104-119	Promotes calcium uptake	1.02E+08	1.35E+09	2.78E+07	Phosphorylation
	SDIPNPIGSENSEK	180-193	Antimicrobial	3.90E+08	1.11E+07	1.02E+09	Unmodified, Phosphorylation, Lactosylation
α_{s2}-casein	NMAINPSK	25-32	ACE-inhibitory	6.02E+07	3.82E+07	3.18E+07	Unmodified, Phosphorylation
	ALNEINQFYQK	81-91	ACE-inhibitory		6.99E+06		Unmodified
	VPITPT	117-122	DPP-IV Inhibitory	3.34E+08	1.35E+08	3.28E+08	Unmodified
β-casein	LNVPGEIVE	6-14	ACE-inhibitory	3.58E+07		3.05E+07	Unmodified
	NVPGEIVESL	7-16	Antioxidant	7.39E+07	9.53E+07	1.01E+08	Phosphorylation
	FQSEEQQQTEDELQDK	33-48	Promotes calcium uptake	9.68E+08	8.37E+08	1.16E+09	Unmodified, Phosphorylation, Deamidation
	FQSEEQQQTEDELQDKIHPF	33-52	Promotes calcium uptake	6.00E+06	3.83E+07		Phosphorylation
	VYPFPGPI	59-66	PEP-inhibitory	6.92E+07	4.91E+07	3.63E+07	Unmodified
	VYPFPGPIPN	59-68	Antioxidant, ACE-inhibitory	4.31E+09	3.28E+09	3.22E+09	Unmodified, Deamidation
	YPFPGPI	60-66	Satiety, Reduces pancreas MDA level, Opioid, Increases MUC5A expression, Increases MUC3 expression, Increases MUC2 expression, Increases jejunal mucus secretion, immunomodulatory, Anxiolytic, Anticancer, ACE-inhibitory, Antioxidant	9.30E+07	1.36E+08	4.74E+07	Unmodified
	YPFPGPIPN	60-68	DPP-IV Inhibitory, ACE-inhibitory, Antioxidant	3.25E+08	3.76E+08	1.68E+08	Unmodified
	YPFPGPIPNS	60-69	Anti-anxiety	2.61E+07	1.13E+07	5.94E+06	Unmodified
	PFPGPIPN	61-68	ACE-inhibitory	9.53E+08	7.58E+08	5.08E+08	Unmodified
	FPGPIPN	62-68	DPP-IV Inhibitory	1.74E+06	5.30E+06		Unmodified

	PGPIPN	63-68	immunomodulatory, Anticancer, Anti-inflammatory, ACE-inhibitory	6.20E+07	4.44E+07	5.71E+07	Unmodified
	SLPQNIPPL	69-77	DPP-IV Inhibitory	2.07E+08	1.36E+08	1.82E+08	Unmodified
	SLPQNIPPLTQTPVVVPPF	69-87	Anticancer	1.24E+08	1.08E+08	1.12E+08	Unmodified
	LPQNIPPLT	70-78	DPP-IV Inhibitory	6.06E+06	3.61E+06	5.85E+06	Unmodified
	NIPPLTQTPV	73-82	ACE-inhibitory	6.66E+08	1.58E+09	4.72E+08	Unmodified
	IPPLTQT	74-80	DPP-IV Inhibitory	3.11E+07	1.10E+07	4.03E+07	Unmodified
	TPVVVPPFLQP	80-90	ACE-inhibitory	5.35E+07	2.01E+07	9.39E+06	Unmodified
	HKEMPFPK	106-113	Antimicrobial	2.67E+09	1.41E+09	5.44E+08	Unmodified, CML
	FPKYPVEPF	111-119	Antioxidant	2.49E+07	2.85E+07	1.94E+07	Unmodified
	YPVEPF	114-119	Opioid, Increases MUC4 expression, DPP-IV Inhibitory, Antioxidant, Antimicrobial	7.02E+09	6.48E+09	4.93E+09	Unmodified
	DVENLHLPLPL	129-139	Antimicrobial	2.98E+06	1.82E+07	1.04E+07	Unmodified
	WMHQPHQPLPPT	143-154	Anti-inflammatory, ACE-inhibitory	3.26E+09	8.51E+08	3.19E+09	Unmodified
	HQPHQPLPPT	145-154	ACE-inhibitory	3.78E+09	1.52E+09	2.11E+09	Unmodified
	HQPHQPLPPTVMFPPQ	145-160	Anti-inflammatory, ACE-inhibitory	5.74E+06	1.12E+08		Unmodified
	SQSKVLPVPQ	166-175	ACE-inhibitory			1.78E+07	CML
	SKVLPVPQ	168-175	ACE-inhibitory	2.10E+07	8.81E+06	1.32E+07	Unmodified
	LYQEPVLGPVR	192-202	Anti-inflammatory, ACE-inhibitory	3.40E+07	9.52E+07	3.80E+07	Unmodified
	YQEPVLGPVR	193-202	ACE-inhibitory, Immunomodulatory, Antithrombotic, Antioxidant, Anti-inflammatory	8.83E+07	2.57E+07	1.19E+08	Unmodified
	YQEPVLGPVRGPFPIIV	193-209	ACE-inhibitory, Immunomodulatory, Antithrombotic, Antioxidant, Anticancer	3.35E+06	3.76E+06	1.22E+07	Unmodified
	QEPVLGPVRGPFPIIV	194-209	ACE-inhibitory		8.40E+07		Unmodified
κ-casein	YPSYGLN	35-41	Opioid		4.48E+07	2.51E+07	Unmodified
	YYQQKPVA	42-49	Antimicrobial	7.25E+08	1.22E+08	8.25E+08	Unmodified, CML
	MAIPPKKNQDKTEIPTINT	106-124	Antimicrobial			1.72E+07	Lactosylation
β-Lg	LIVTQTMK	1-8	Cytotoxic	3.43E+08	3.71E+07	1.25E+08	Unmodified
	GLDIQKVAGT	9-18	Antimicrobial	2.08E+08	5.91E+06	8.86E+09	Unmodified, Deamidation, Lactosylation, CML
	DAQSAPLRVY	33-42	ACE-inhibitory			4.08E+07	Unmodified
	VYVEELKPTPEGDLEILLQK	41-60	Hypocholesterolemic	5.65E+06	8.71E+05	7.24E+06	Unmodified

	LKPTPEGDL	46-54	DPP-IV Inhibitory	2.33E+07	4.24E+08	1.84E+07	Unmodified
	LKPTPEGDLE	46-55	DPP-IV Inhibitory	2.67E+07	3.18E+08	1.65E+07	Unmodified
	IDALNENK	84-91	Stimulates proliferation, Antimicrobial	1.13E+08	1.37E+08	1.41E+08	Unmodified
	VLVLDTDYK	92-100	DPP-IV Inhibitory, Antimicrobial	8.06E+08	1.47E+08	5.46E+08	Unmodified, Lactosylation
	VLDTDYK	94-100	ACE-inhibitory	6.66E+09	1.30E+09	8.25E+09	Unmodified, Lactosylation
	TPEVDDEALEK	125-135	DPP-IV Inhibitory, Antimicrobial	3.74E+10	1.92E+10	3.69E+10	Unmodified, Lactosylation, CML
	ALPMHIR	142-148	Stimulates proliferation, Reduces vasoconstrictor endothelin-1 release, ACE-inhibitory	1.74E+08	1.63E+07	6.88E+07	Unmodified
	LSFNPTQ	149-155	ACE-inhibitory	1.70E+07		2.11E+07	Deamidation
α-La	GYGGVSLPEW	17-26	ACE-inhibitory	1.89E+08		2.52E+08	Unmodified
	VSLPEW	21-26	ACE-inhibitory	3.68E+08	7.46E+08	4.59E+08	Unmodified
	IWCKDDQNPH	59-68	Antioxidant		2.51E+06		Unmodified
	ILDKVGINY	95-103	DPP-IV Inhibitory		3.24E+07		Unmodified
Count				**49**	**51**	**50**	
Total Intensity				**7.35E+10**	**4.25E+10**	**7.60E+10**	

Table S5: Protein concentrations of intestinal digests before and after filtration of the digests by centrifugal filtration with a 10 kDa molecular weight cut off. An infant formula model system remained unheated (UH) and was digested by use of *an in vitro* infant digestion model. Water instead of the infant formula model system was digested with the *in vitro* digestion model to obtain a control digest.

	UH	**Control digest**	**Δ UH – Control digest**
Protein concentration before filtration (mg/ml)	6.47	2.60	3.87
Protein concentration after filtration (mg/ml)	3.91	1.25	2.66
Protein recovery (%)	60	48	69

Table S6: HLA-II epitopes predicted by the use of the IEDB MHC Class II Binding Prediction tool at the basolateral side after transport across a 21-days differentiated Caco-2 or Caco-2/HT29-MTX-E12 (90/10) monolayer. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells for 6h. Amino acid (AA) position provides the position of the peptide containing an HLA epitope in the protein without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form. ND: not detected.

Protein of origin	**Peptide**	**AA position**	**HLA gene**	**Intensity Caco-2**			**Intensity Caco-2 & HT29-MTX-E12**			**Modification**
				UH	**WH-40**	**DH-72**	**UH**	**WH-40**	**DH-72**	
α_{S1}-casein	LEIVPNSAEER	109-119	HLA-DR				1.33E+06	5.13E+06		Unmodified, Phosphorylation
α_{S2}-casein	NMAINPSKENL	25-35	HLA-DR				4.42E+05			Phosphorylation
	QGPIVLNPWDQVK	101-113	HLA-DR, HLA-DP						7.55E+05	Lactosylation
β-casein	PVVVPPFLQPE	81-91	HLA-DP				2.92E+05		2.79E+05	Unmodified
	SLSQSKVLPVPQ	164-175	HLA-DR, HLA-DQ	3.83E+05		1.13E+07		6.34E+05	3.90E+07	Lactosylation, CML
β-Lg	EVDDEALEKFD	127-137	HLA-DQ	4.96E+06		2.55E+06	1.65E+07	1.49E+06	8.28E+06	Unmodified
GRIP and coiled-coil domain containing 2	IDQLKLKLQDTQNS	1358-1371	HLA-DR				1.47E+06		6.29E+05	Deamidation, CML
Count				**2**	**ND**	**2**	**5**	**3**	**5**	
Total Intensity				**5.35E+06**	**ND**	**1.38E+07**	**2.00E+07**	**7.25E+06**	**4.90E+07**	

Table S7: Linear IgE epitopes predicted at the basolateral side after transport across a 21-days differentiated Caco-2 or Caco-2/HT29-MTX-E12 (90/10) monolayer. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells for 6h. Amino acid (AA) position provides the position of the peptide containing an HLA epitope in the protein without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form. ND: not detected.

Protein of origin	**Peptide**	**AA position**	**IgE epitope position**	**Intensity Caco-2**			**Intensity Caco-2 & HT29-MTX-E12**			**Modification**
				UH	**WH-40**	**DH-72**	**UH**	**WH-40**	**DH-72**	
α_{S1}-casein	LEIVPNSAEER	109-119	109-120				1.33E+06	5.13E+06		Unmodified, Phosphorylation
	EIVPNSAEER	110-119	109-120	3.19E+07			1.30E+08	3.22E+07	1.91E+08	Unmodified, Phosphorylation
	TDAPSFSDIPNPI	174-186	171-185					7.51E+06		Unmodified
α_{S2}-casein	QGPIVLNPWDQVK	101-113	105-114						7.55E+05	Lactosylation
β-casein	QSLVYPFPGPIPN	56-68	55-70						8.13E+05	Unmodified
	SLPQNIPPLTQTPVVVPPFLQPE	69-91	83-92				2.78E+05			Unmodified
	SLPQNIPPLTQTPVVVPPFLQPEV	69-92	83-92					2.08E+06	4.11E+06	Unmodified
	NIPPLTQTPVVVPPFLQPE	73-91	83-92				3.17E+05	4.46E+05	5.06E+05	Unmodified
	NIPPLTQTPVVVPPFLQPEV	73-92	83-92				8.16E+06	9.57E+06	1.06E+07	Unmodified
	NIPPLTQTPVVVPPFLQPEVMG	73-94	83-92						2.11E+05	Unmodified
	IPPLTQTPVVVPPFLQPEV	74-92	83-92				4.04E+05	4.16E+05	7.85E+05	Unmodified
	TQTPVVVPPFLQPEV	78-92	83-92	4.64E+05			9.99E+05	8.54E+05	9.66E+05	Unmodified
	QTPVVVPPFLQPEV	79-92	83-92				8.33E+05	8.46E+05	1.52E+06	Unmodified
	PVVVPPFLQPE	81-91	83-92				2.92E+05		2.79E+05	Unmodified
	PVVVPPFLQPEV	81-92	83-92	1.58E+07			4.58E+07	1.00E+07	6.74E+07	Unmodified
	PVVVPPFLQPEVMG	81-94	83-92						5.54E+05	Unmodified
	VVVPPFLQPEV	82-92	83-92						1.27E+06	Unmodified
	PPFLQPEV	85-92	83-92				4.76E+05		6.78E+05	Unmodified
	HQPHQPLPPTVMFPPQS	145-161	149-164					2.92E+05		Unmodified
	HQPHQPLPPTVMFPPQSV	145-162	149-164					5.15E+06		Unmodified
β-Lg	VRTPEVDDEALEKFDK	123-138	121-140				3.29E+06			Unmodified

Count	**3**	**ND**	**ND**	**12**	**12**	**15**
Total Intensity	**4.82E+07**	**ND**	**ND**	**1.92E+08**	**7.44E+07**	**2.82E+08**

Table S8: Bioactive peptides identified by use of the milk bioactive peptide database (MBPDB) at the basolateral side after transport across a 21-days differentiated Caco-2 or Caco-2/HT29-MTX-E12 (90/10) monolayer. An infant formula model system remained either unheated (UH), was wet heated for 40 min (WH-40) or dry heated for 72h (DH-72) and all samples were digested by use of an *in vitro* infant digestion model and filtered before the intestinal digests were applied to the Caco-2 and HT29-MTX-E12 cells for 6h. Amino acid (AA) position provides the position of the peptide containing an HLA epitope in the protein without the signal peptide. Modification indicates if the peptide was detected in the digest in unmodified, phosphorylated, deamidation modified, lactosylated, or carboxymethyllysine (CML) modified form.

Protein of origin	**Peptide**	**AA position**	**Bioactive function**	**Intensity Caco-2**			**Intensity Caco-2 & HT29-MTX-E12**			**Modification**
				UH	**WH-40**	**DH-72**	**UH**	**WH-40**	**DH-72**	
α_{S1}-casein	SDIPNPIGSENSEK	180-193	Antimicrobial				7.30E+05			Unmodified
β-casein	FQSEEQQQTEDELQDK	33-48	Promote calcium uptake	5.73E+06	1.41E+06	4.65E+06	3.71E+06	1.03E+06	3.57E+06	Unmodified, Phosphorylation
	VYPFPGPIP	59-67	PEP-inhibitory	1.96E+06			4.98E+06	2.72E+06	6.99E+06	Unmodified
	SLPQNIPPLTQTPVVVPPF	69-87	Anticancer					6.37E+05	1.00E+06	Unmodified
	NIPPLTQTPV	73-82	ACE-inhibitory				2.34E+06	3.09E+06	2.07E+06	Unmodified
	IPPLTQT	74-80	DPP-IV Inhibitory				4.61E+05		6.92E+05	Unmodified
	YPVEPF	114-119	Opioid, Increases MUC4 expression, DPP-IV Inhibitory, Antioxidant, Antimicrobial					1.56E+06		Unmodified
	WMHQPHQPLPPT	143-154	Anti-inflammatory, ACE-inhibitory	8.16E+05	2.43E+05	6.73E+05	1.71E+06	4.47E+05	3.90E+06	Unmodified
	HQPHQPLPPT	145-154	ACE-inhibitory	2.50E+07	3.57E+06	1.58E+07	4.42E+07	8.64E+06	5.85E+07	Unmodified
	SQSKVLPVPQ	166-175	ACE-inhibitory						5.29E+06	Lactosylation
	VLPVPQ	170-175	Inhibition of cholesterol solubility	2.47E+07		1.96E+07	5.64E+07	2.85E+07	5.84E+07	Unmodified
	QEPVLGPVRGPFPIIV	194-209	ACE-inhibitory					3.00E+05		Unmodified
β-Lg	GLDIQKVAGT	9-18	Antimicrobial	8.30E+05			2.12E+06	4.45E+05	1.24E+08	Lactosylation, CML
	VLVLDTDYK	92-100	DPP-IV Inhibitory, Antimicrobial				1.19E+06		4.49E+05	Unmodified

	VLDTDYK	94-100	ACE-inhibitory	1.08E+07			2.28E+07	9.60E+05	5.16E+07	Unmodified, Lactosylation
	TPEVDDEALEK	125-135	DPP-IV Inhibitory, Antimicrobial	4.89E+07	8.63E+05	2.15E+07	8.45E+07	1.33E+07	7.43E+07	Unmodified, Lactosylation, CML
α-La	GYGGVSLPEW	17-26	ACE-inhibitory						1.94E+05	Unmodified
	VSLPEW	21-26	ACE-inhibitory				9.37E+05	1.24E+06	2.78E+06	Unmodified
Count				**8**	**4**	**5**	**13**	**13**	**15**	
Total Intensity				**1.19E+08**	**6.08E+06**	**6.23E+07**	**2.26E+08**	**6.30E+07**	**3.94E+08**	

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